

ASPECTS ON SISTER CHROMATID EXCHANGE IN HUMAN LYMPHOCYTES

by
Karin Hedner



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Abstract Sister chromatid exchanges (SCE) and structural chromosome aberrations were analyzed in peripheral blood lymphocytes of 100 individuals. Of these, 65 were occupationally exposed, and 35 were healthy unexposed controls. There was no significant correlation between SCE frequency and any of the different types of structural chromosome aberrations recorded within each of the different exposure groups, among the controls, or in the total material. There was no SCE difference between smokers and nonsmokers in the total material. Among individuals exposed to epoxy resins, though, smokers had significantly more SCE ($P < 0.05$) than nonsmokers. There was no correlation between SCE frequency and age. However, females had higher values than males ($P < 0.01$). SCE frequencies were analyzed in 18 male twin pairs, 9 monozygotic (MZ) and 9 dizygotic (DZ), before, 1 h after and 18 h after in vitro treatment with N-acetoxy-2-acethylaminofluorene (NA-AAF). There was no significant difference in the intraclass correlation coefficients of SCE frequencies among MZ and DZ pairs, indicating that genetic factors do not have a major influence on spontaneous or carcinogen induced SCE frequencies. Thus, any observed increase in SCE frequencies is probably caused by environmental influence. SCE frequencies were analyzed in 15 healthy blood donors before, 1 h after and 18 h after in vitro treatment with EO. There was a significant decrease in mean SCE frequency after 18 h of DNA repair incubation as compared to that after 1 h of EO treatment ($P < 0.001$), indicating that a significant portion of the EO induced DNA damage had been repaired. In contrast, there was no significant decrease in mean SCE frequency of NA-AAF exposed lymphocytes after 18 h of DNA repair incubation, suggesting that only a small portion of the NA-AAF induced damage had been removed. EO induced DNA damage seems likely to be more easily recognized by cellular repair enzymes than NA-AAF induced damage. The reason for this may be the different chemical nature of the DNA lesions induced, leading to a different kinetics of DNA repair.			
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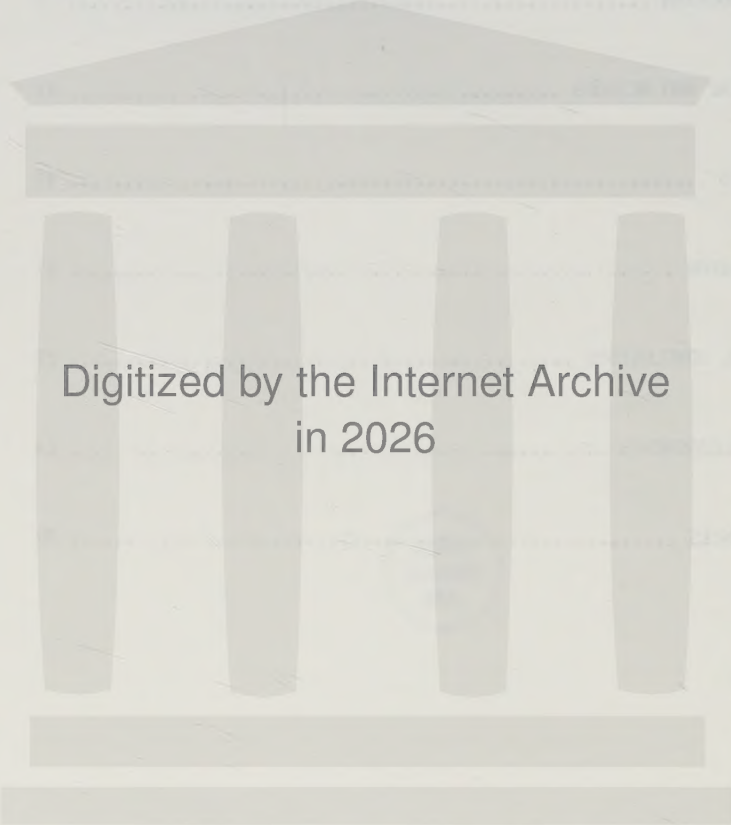


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This thesis is based on the following papers, referred to by their

Roman numerals:

- I Hedner K, Högstedt B, Kolnig A-M, Mark-Vendel E, Strömbeck B, and Mitelman F (1982) Relationship between sister chromatid exchanges and structural chromosome aberrations in lymphocytes in 100 individuals. *Hereditas* 97:237-245
- II Mitelman F, Fregert S, Hedner K, and Hillbertz-Nilsson K (1980) Occupational exposure to epoxy resins has no cytogenetic effect. *Mutat Res* 77:345-348
- III Högstedt B, Gullberg B, Hedner K, Kolnig A-M, Mitelman F, Skerfving S, and Widegren B (1983) Chromosome aberrations and micronuclei in bone marrow cells and peripheral blood lymphocytes in humans exposed to ethylene oxide. *Hereditas* 98:105-113
- IV Hedner K, Högstedt B, Kolnig A-M, Mark-Vendel E, Strömbeck B, and Mitelman F (1983) Sister chromatid exchange and structural chromosome aberrations in relation to smoking in 91 individuals. *Hereditas* 98:77-81
- V Hedner K, Högstedt B, Kolnig A-M, Mark-Vendel E, Strömbeck B, and Mitelman F (1982) Sister chromatid exchanges and structural chromosome aberrations in relation to age and sex. *Hum Genet* 62:305-309

- VI Hedner K, Mitelman F, Nordén Å, and Pero RW. A twin study of sister chromatid exchanges in human lymphocytes following carcinogen exposure and DNA repair incubation. Submitted to Hum Genet
- VII Hedner K, Mitelman F, and Pero RW. Sister chromatid exchanges in human lymphocytes after a non-S-phase incubation period to allow excision DNA repair - in vitro exposure to N-acetoxy-2-acetylaminofluorene and ethylene oxide. Submitted to Mutat Res

INTRODUCTION

Analysis of structural chromosome aberrations in peripheral blood lymphocytes is the only method, which so far has been practically useful in detecting genetic damage directly in man. However, the absence of chromosome aberrations does not exclude other types of DNA damage. Also, the method is laborious, time-consuming, and requires a high degree of skill on the part of the scorer, which makes the capacity for extensive analyses in existing laboratories limited. Therefore, the demonstration of sister chromatid exchanges (SCE) has gained much interest as a potentially more sensitive indicator of damage to the genetic material. SCE involve equal symmetrical exchange at one locus between two sister chromatids, and do not result in any alteration of overall chromosome morphology. Thus, previously undetectable damage to the genetic material might in this way be revealed.

SCE were observed for the first time by Taylor et al. (1957), who produced the first autoradiographs of metaphase chromosomes with tritium-labelled thymidine. Since then, techniques have been developed to visualize these exchange events without the use of autoradiography. Addition of a DNA base analogue, for instance 5-Bromodeoxyuridine (BrdU), to the culture results in alteration of the fluorescence of added DNA-binding dyes, such as 33 258 Hoechst. This procedure makes the SCE readily visible (Latt 1973, Perry and Wolff 1974).

The biological significance of SCE is to a large extent unknown. There

is no doubt that SCE is a sensitive indicator of chemically induced damage to the genetic material in vitro. In fact, significant increases in SCE have been induced in cultured cells by a number of chemicals at levels that produce little or no chromosome change (Latt 1974, Perry and Evans 1975, Kato and Shimada 1975, Wolff 1977, Raposa 1978). Considerably less information is, however, available on the SCE frequency in normal healthy human subjects, and after chronic in vivo exposure to potential mutagens. Moreover, considerable variation in the SCE frequencies has been found among the individuals studied (Lambert et al. 1976, Kurvink et al. 1978, Bala Krishna Murthy and Prema 1979, Morgan and Crossen 1981, Carrano and Moore 1982), ranging from 5.8 to 22.2. The reasons for this may be different culture conditions and/or influences from unrecognized environmental, constitutional, and genetic factors.

It is known that the demonstration of SCE is influenced by a variety of technical factors. Although it can be presumed that SCE occur spontaneously, a considerable proportion of them is induced by BrdU itself (Wolff and Perry 1974, Galloway and Evans 1975, Lambert et al. 1976). The response to BrdU may also be complex (Latt and Juergens 1977). Other factors too exert an influence, for instance the composition of the medium, especially the serum (Kato and Sandberg 1977), the staining method (Morgan and Crossen 1981), light (Ikushima and Wolff 1974, Monticone and Schneider 1979), temperature (Speit 1980), and the rate of cell proliferation (Lindblad and Lambert 1981).

A widespread environmental factor, that may cause cytogenetic damage in

man, is cigarette smoking. However, the reports on this aspect are contradictory. An increased rate of SCE in smokers has been reported by several authors (Lambert et al. 1978, Bala Krishna Murthy 1979, Husgafvel-Pursiainen et al. 1980, Husum et al. 1980, Carrano and Moore 1982), while others have found a normal SCE rate in moderate as well as heavy smokers (Hollander et al. 1978, Crossen and Morgan 1980, Ardito et al. 1980, Waksvik et al. 1981a).

Two obvious constitutional factors, that may influence the frequency of cytogenetic damage are the age and the sex of the individual. Many previous reports have not found any correlation between the SCE rate and age in adults (Galloway and Evans 1975, Funes-Cravioto et al. 1977, Hollander et al. 1978, Carrano and Moore 1982), but recently higher SCE rates have been observed in older individuals (Goh 1981, Schmidt and Sanger 1981, Waksvik et al. 1981a). Children are reported to have fewer SCE than adults (Funes-Cravioto et al. 1977, Ardito et al. 1980, Husgafvel-Pursiainen et al. 1980, Schmidt and Sanger 1981).

The relationship between sex and cytogenetic damage has been investigated by several authors, but no difference in SCE rates has been noted (Galloway and Evans 1975, Latt and Juergens 1977, de Arce 1981, Waksvik et al. 1981a, Husum et al. 1982, Carrano and Moore 1982). It may be mentioned, though, that in several of these studies the values for females were in fact higher than those for males, but apparently the differences were not statistically significant.

The possibility of a genetically determined variation in the SCE frequency may be considered. In studies of twins no difference in the variation of spontaneous SCE frequencies has been found between monozygotic (MZ) and dizygotic (DZ) pairs (Pedersen et al. 1981, Waksvik et al. 1981a, Lambert et al. 1982). This apparent lack of genetically determined baseline SCE frequency does not exclude the possibility of a genetically determined difference in the response to exposure to mutagenic agents. This aspect may be studied by analyzing SCE rates following in vitro carcinogen treatment of lymphocytes of MZ and DZ twin pairs.

Finally, the effect of DNA repair synthesis on the induction of SCE may be considered. The induction of SCE is S-phase dependent, and hence, it appears to be closely related to DNA replication (Wolff 1982). Although SCE are likely to be related to DNA repair processes, their relationship to repair replication, unscheduled DNA synthesis, and postreplication repair remains unclear (Kato 1977). Peripheral lymphocytes are mainly in G₀-phase of the cell cycle (Clarkson and Evans 1972), and it can be easily shown that excision DNA repair synthesis occurs in these cells (Lieberman et al. 1971a, Lieberman et al. 1971b). Therefore, if lymphocytes are cultured after DNA damage induction, they would be allowed to remove DNA damage by excision repair before entering S-phase, following the addition of mitogen. In S-phase, then, there would be less DNA damage present to induce SCE. This has been reported to be the case in experiments with chinese hamster ovary (CHO) cells (McRae et al. 1979).

AIMS OF THE INVESTIGATION

The aims of the present investigation were

- to determine the SCE variation in a healthy reference population
- to study the relationship between SCE and structural chromosome aberrations
- to evaluate the possible influence on SCE of environmental and constitutional factors, such as cigarette smoking, age and sex
- to evaluate the possible influence on SCE of genetic factors
- to evaluate the effect of DNA repair synthesis on the induction of SCE

MATERIAL AND METHODS

The following individuals were investigated:

100 individuals, 69 males aged 18-65 years (mean 36.4), and 31 females aged 18-58 years (mean 38.9) (Papers I and V).

22 individuals (Cases 1-22), 8 males aged 20-55 years (mean 33.0), and 14 females aged 19-54 years (mean 39.1) were occupationally exposed to ethylene oxide (EO), and were described separately in Paper III.

17 individuals (Cases 23-39), 13 males aged 21-65 years (mean 40.7), and 4 females aged 18-58 years (mean 34.0) were occupationally exposed to epoxy resins, and were described separately in Paper II.

13 individuals (Cases 40-52), all males aged 18-56 years (mean 31.8), were occupationally exposed to a variety of organic solvents, mainly toluene.

7 individuals (Cases 53-59), 6 males aged 22-44 years (mean 34.8), and 1 female aged 25 years, were bus drivers, and were occupationally exposed to petrol products.

6 individuals (Cases 60-65), all males aged 32-51 years (mean 43.5), were suffering from advanced chronic alcoholism.

35 individuals (Cases 66-100), 23 males aged 20-59 years (mean 35.6), and 12 females aged 21-58 years (mean 41.8), were healthy unexposed factory workers.

91 of the 100 individuals, 62 males aged 20-65 years (mean 37.4), and 29 females aged 18-58 years (mean 38.4), were analyzed separately with regard to their smoking habits (Paper IV).

18 male twin pairs, 9 MZ and 9 DZ, aged 25-33 years (mean 29.1), were investigated regarding their SCE frequencies before, 1 h after and 18 h after in vitro treatment with a $100\text{ }\mu\text{mol/l}$ dose of N-acetoxy-2-acetylaminofluorene (NA-AAF) for 1 h at 37°C (Papers VI and VII).

15 healthy blood donors, 11 males and 4 females, were investigated regarding their SCE frequencies before, 1 h after and 18 h after in vitro treatment with a 2 mmol/l dose of EO for 1 h at 37°C (Paper VII).

The frequency of SCE was analyzed in PHA-stimulated peripheral blood lymphocytes cultured for 72 h and studied with Giemsa-technique essentially according to Wolff and Perry (1974) as described in detail in Paper I. BrdU was present during the entire culture period at a final concentration of $100\text{ }\mu\text{mol/l}$ medium. 20 metaphases (whenever possible) with 46 chromosomes from each individual were analyzed on coded slides.

The frequency of structural chromosome aberrations was analyzed in PHA-stimulated peripheral blood lymphocytes cultured for 72 h by a

standard microculture technique. The procedure is described in detail in Paper I. 200 metaphases from each individual were analyzed on coded slides.

The statistical methods used were the Pearson correlation coefficient test (Papers I, IV, and V), Student's t-test (Papers II, VI, and VII), the Spearman rank correlation test (Paper III), analysis of covariance (Papers III, IV, and V), F-test and one-way analysis of variance (Paper VI). Heritability (h^2) estimates were made by the formula $h^2 = 2(r_{MZ} - r_{DZ})$, where r_{MZ} and r_{DZ} are the sample intraclass correlation coefficients among MZ and DZ twins, respectively (Weinberg et al. 1976) (Paper VI).

RESULTS

SCE variation in a healthy reference population

The mean frequency of SCE in the 100 individuals studied (Paper I) was 9.4 (S.D.=2.3). In the combined exposure groups (Cases 1-65) it was 9.6 (S.D.=2.6), and in the control subjects (Cases 66-100) it was 9.0 (S.D.=1.4). The mean frequency of SCE among the 36 healthy unexposed twins was 8.9 (S.D.=1.5) (Paper VI). The mean frequency of SCE among the 15 healthy blood donors was 9.7 (S.D.=1.3) (Paper VII). In the pooled group of healthy unexposed persons (n=86) the mean SCE frequency was 9.1 (S.D.=1.5). In the pooled group of all individuals studied (n=151) the mean SCE frequency was 9.3 (S.D.=2.1).

Relationship between SCE and structural chromosome aberrations

There was no significant correlation between SCE frequency and any of the different types of structural chromosome aberrations recorded (gaps, breaks, and exchange types of aberrations) in the 100 individuals studied (Paper I). The lack of correlation was evident within each of the different exposure groups, among the control subjects, as well as in the total material.

Influence on SCE of environmental and constitutional factors

There was no difference between smokers and nonsmokers as regards the SCE frequency in the total material. Among individuals exposed to epoxy resins, though, smokers had significantly more SCE ($P < 0.05$) than nonsmokers. There was no difference between smokers and nonsmokers regarding the frequency of structural chromosome aberrations in the total material, nor in any of the subgroups (Paper IV).

No significant correlation was found between the SCE rate and age. However, such a correlation was found between the frequency of structural chromosome aberrations and age. Thus, older individuals had significantly more aberrations than younger. This was true for gaps ($P < 0.05$), breaks ($P < 0.05$), exchanges ($P < 0.05$), and total No. of aberrations ($P < 0.01$) (Paper V).

There was a significant sex difference in SCE rates. Females had higher values than males ($P < 0.01$). Females also had significantly more structural chromosome aberrations than males. This was true for breaks ($P < 0.01$), exchanges ($P < 0.01$), and total No. of aberrations ($P < 0.01$) (Paper V).

With the aid of covariance analysis it was possible to show that the correlations between the different types of cytogenetic damage and age and sex, respectively, were not interrelated, nor could they be explained by different smoking habits.

Influence on SCE of genetic factors

There was no significant difference in the intraclass correlation coefficients of SCE frequencies among MZ and DZ twin pairs at the baseline level, nor after NA-AAF exposure or following a DNA repair incubation period. Neither was there any significant difference in the intraclass variation as regards the difference between the baseline level of SCE rates and the NA-AAF induced SCE rates, between the NA-AAF induced SCE rates and the SCE rates after DNA repair incubation, nor between the baseline level and the SCE rates after DNA repair incubation. h^2 estimates resulted in values of 0 at the baseline level, -1.16 after NA-AAF exposure, 0.32 after DNA repair incubation, -1.84 for the difference between the baseline level and the NA-AAF induced SCE rates, 0.04 for the difference between the NA-AAF induced SCE rates and the SCE rates after DNA repair incubation, 0.48 for the difference between the baseline level and the SCE rates after DNA repair incubation (Paper VI).

Effect of DNA repair synthesis on the induction of SCE by DNA damaging agents with different kinetics

There was a significant difference between the baseline SCE frequencies and the SCE frequencies after 1 h of NA-AAF treatment ($P < 0.001$), and also between the baseline frequencies and the SCE frequencies after 18 h of DNA repair incubation ($P < 0.001$). However, there was no significant difference between the SCE frequencies after 1 h of NA-AAF treatment and

the SCE frequencies after 18 h of DNA repair incubation.

There was a similar significant difference between the baseline SCE frequencies and the SCE frequencies after 1 h of EO treatment ($P < 0.001$), and also between the baseline SCE frequencies and the SCE frequencies after 18 h of DNA repair incubation ($P < 0.001$). Moreover, there was a significant decrease in the mean SCE rate after 18 h of DNA repair incubation as compared to the mean SCE rate after 1 h of EO treatment ($P < 0.001$) (Paper VII).

DISCUSSION

A close correlation between chemicals with mutagenic activity in Ames' test and carcinogenic properties in experimental animals has been reported (McCann and Ames 1976), leading to the conclusion that mutagens can also be carcinogens. This has urged the need for methods to determine mutagenic effects directly in man. So far, the only way to do this has been by the analysis of structural chromosome aberrations. Recently, however, a number of other methods have been developed, such as SCE, micronuclei, and unscheduled DNA synthesis. This thesis has been concerned with the SCE method, its possible applications and limitations.

In the population studied, the variation between mean SCE frequencies in the different groups was small (8.9-9.7). The wide range of mean SCE frequencies previously found in various healthy human populations is to some extent caused by different culture conditions. For instance, the final concentration of BrdU varied from 10 $\mu\text{mol/l}$ to 100 $\mu\text{mol/l}$ in the reports referred to in the Introduction. However, there is no clear dose-response curve, indicating that other variations in the culture conditions also may contribute to the differences found. As long as the techniques used in different laboratories vary, a general reference value for the mean number of SCE in a healthy human population cannot be used, but must always be correlated to the technique used in each laboratory.

Very few chemicals have been investigated as regards their ability to induce SCE after chronic in vivo occupational exposure. Occupational exposure to vinyl chloride monomer (KucEROVÁ et al. 1979) and styrene (HögstEDT et al. 1979, Andersson et al. 1980) has been shown to give rise to an increased SCE rate as well as an increased frequency of structural chromosome aberrations. This was the case also after occupational exposure to a variety of organic solvents (Funes-Cravioto et al. 1977), but in this study no particular chemical agent could be identified as the causative factor. Studies of occupational exposure to different cytostatic drugs among hospital nurses have given controversial results, perhaps because of different exposure times and different routines in handling the drugs (Norppa et al. 1980, Waksvik et al. 1981b, Brögger et al. 1981). Occupational exposure to halothane, nitrous oxide (Husum and Wulf 1980, Waksvik et al 1981b), benzene (Watanabe et al. 1980), inorganic lead (Mäki-Paakkanen et al. 1980a), nickel compounds (Waksvik and Boysen 1982), tetrachloroethylene (Ikeda et al. 1980), toluene and xylene (Mäki-Paakkanen et al. 1980b, Haglund et al. 1980) has not been found to give rise to increased SCE rates, although exposure to nickel compounds gave an increased frequency of structural chromosome aberrations.

In the two exposure groups that we have studied (epoxy resins and EO) there was no increase in SCE rates compared to controls, although EO exposure gave an increased frequency of structural chromosome aberrations. Thus, our results are in accordance with the suggestion that the mechanisms leading to the formation of SCE are heterogenous and fundamentally different from those that cause structural chromosome aberrations (Gebhart 1981).

This conclusion is also supported by the fact that some agents producing severe chromosome breakage, such as X-rays, have little or no effect on SCE frequency (Perry and Evans 1975). Also, among patients with hereditary chromosome instability disorders, such as ataxia telangiectasia, Bloom's syndrome, and Fanconi's anemia, characterized by a markedly increased frequency of spontaneous chromosome aberrations, only patients with Bloom's syndrome display an increase in SCE (Chaganti et al. 1974), while patients with ataxia telangiectasia and Fanconi's anemia have a normal frequency (Galloway and Evans 1975, Latt et al. 1975).

A chromosome damaging effect of smoke condensates in vitro has been shown (Hopkin and Evans 1979), and an increased frequency of chromosome aberrations has been demonstrated in heavy smokers (Obe and Herha 1978). The fact that only among individuals exposed to epoxy resins, smokers had more SCE than nonsmokers, is an intriguing one. It indicates a complex relationship between smoking and cytogenetic damage, and urges the need for further data correlating smoking habits to different types of cytogenetic damage in different populations.

Only one previous study has reported a correlation between structural chromosome aberrations and age in adults (Tough et al. 1970). Concerning structural chromosome aberrations in children there are contradictory results. One study reports fewer chromosome aberrations in children than in adults (Funes-Cravioto et al. 1977), whereas in another study an increased frequency of chromosome aberrations was found in neonates, falling to adult values within the first two years (Sasaki et al. 1970).

Our finding that the SCE rate in vivo was not correlated to the age of the subject is in accordance with several previous reports (see Introduction). However, a significant increase in the spontaneous SCE frequency was observed during long term culture at the end of the life span of in vitro cultures of normal human fibroblasts (Kato and Stich 1976). On the other hand, when SCE frequencies were studied in human fibroblast cultures after exposure to mitomycin C and NA-AAF, significantly fewer SCE were found in cultures from old subjects than in those from young subjects at equal levels of early in vitro passage (Schneider and Gilman 1979).

No sex difference has been noted for structural chromosome aberrations (Gebhart et al. 1980). The cause(s) of the sex difference for SCE that we have observed can only be speculated upon at present. An increased SCE rate has been reported in oral contraceptive users (Bala Krishna Murthy and Prema 1979), and therefore other factors than genetic may be considered.

A genetically determined increased susceptibility and/or a decreased resistance to chromosome damaging effects of environmental agents has been suggested, when chromosome damage was estimated by structural chromosome aberrations (Sasaki et al. 1970) or micronuclei (Countryman and Heddle 1976). The effects on different cytogenetic parameters may be entirely different, though. Our twin study indicates that genetic variance does not have a major influence on the variation of spontaneous SCE frequencies, which is in accordance with other twin studies (see

Introduction). We also found an apparent lack of major genetic influence on SCE rates following carcinogen exposure and DNA repair synthesis. Thus, it would seem that SCE is a cytogenetic method well suited to the investigation of chronic in vivo exposure to possible mutagens, since any observed increase in SCE frequencies is probably caused by environmental influence.

We found an obvious decrease in the mean SCE frequency of EO exposed lymphocytes, when they had been held in non-S-phase for 18 h after EO exposure, indicating that a significant portion of the EO induced DNA damage had been repaired. In contrast, there was no significant decrease in the mean SCE frequency of NA-AAF exposed lymphocytes, when they had been held in non-S-phase for 18 h after NA-AAF exposure, suggesting that only a small portion of the original NA-AAF damage to DNA had been removed. It seems likely, then, that the EO induced DNA damage is more easily recognized and hence more rapidly repaired than the NA-AAF induced damage, as judged by SCE analysis. The reason for this may be the chemical nature of the DNA lesions. EO ethylates mainly the No. 7 position of guanine in DNA, which increases depurination and destabilizes the phosphodiester bond (Ehrenberg and Hussain 1981). DNA depurinates at neutral pH to some extent even without any induced DNA damage, also causing a similar instability (Lindahl and Nyberg 1972). Hence, an efficient recognition and repair of this type of DNA damage is essential to permit fidelity to the genetic code. NA-AAF damages DNA primarily in the No. 8 position of guanine, which does not accelerate instability at this site (Kriek 1974), and thus the repair of these DNA lesions are not likely to be controlled by

the same repair enzyme(s). There may be practical implications of these results. For instance, in the field of occupational health care, in cases of exposure to unrecognized carcinogens, that cause SCE alterations, it may be possible to differentiate carcinogens which induce DNA lesions recognized with difficulty by repair enzymes from those that do not.

GENERAL CONCLUSIONS

The present investigation has given the following main results:

- The mean SCE frequency in human peripheral blood lymphocytes in the reference population studied was 9.1 (S.D.=1.5). There was no correlation between SCE rate and age, but females had significantly higher SCE values than males.
- There was no correlation between the results of the SCE method and those of structural chromosome aberrations, indicating that these two parameters are not related.
- There may be a complex relationship between smoking and SCE frequency.
- Genetic factors do not seem to have a major influence on the spontaneous or induced SCE frequencies.
- Carcinogens, which induce DNA damage more easily recognized by cellular repair enzymes, induce less SCE after a preincubation period in non-S-phase, following DNA damage induction, than carcinogens, which induce DNA damage recognized with more difficulty by the repair enzymes.

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REFERENCES

- Andersson HC, Tranberg EA, Uggla AH, and Zetterberg G (1980) Chromosomal aberrations and sister chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic boat factory. *Mutat Res* 73:387-401
- de Arce MA (1981) The effect of donor sex and age on the number of sister chromatid exchanges in human lymphocytes growing in vitro. *Hum Genet* 57:83-85
- Ardito G, Lamberti L, Ansaldi E, and Ponzetto P (1980) Sister chromatid exchanges in cigarette smoking human females and their newborns. *Mutat Res* 78:209-212
- Bala Krishna Murthy P (1979) Frequency of sister chromatid exchanges in cigarette smokers. *Hum Genet* 52:343-345
- Bala Krishna Murthy P and Prema K (1979) Sister chromatid exchanges in oral contraceptive users. *Mutat Res* 68:149-152
- Brögger A, Klepp O, and Waksvik H (1981) Chromosome analyses of nurses handling cytostatic agents. *Mutat Res* 85:254
- Carrano AV and Moore DH (1982) The rationale and methodology for quantifying sister chromatid exchange in humans. In: Heddle JA (ed)

Mutagenicity: New horizons in genetic toxicology. Academic Press, Inc
pp 267-304

Chaganti RSK, Schonberg S, and German J (1974) A manyfold increase
in sister chromatid exchanges in Bloom's syndrome lymphocytes. Proc
Natl Acad Sci (USA) 71:4508-4512

Clarkson JM and Evans HJ (1972) Unscheduled DNA synthesis in human
leucocytes after exposure to UV light, γ -rays and chemical mutagens.
Mutat Res 14:413-430

Countryman PI and Heddle JA (1976) The production of micronuclei from
chromosome aberrations in irradiated cultures of human lymphocytes.
Mutat Res 41:321-332

Crossen PE and Morgan WF (1980) Sister chromatid exchange in cigarette
smokers. Hum Genet 53:425-426

Ehrenberg L and Hussain S (1981) Genetic toxicity of some important
epoxides. Mutat Res 86:1-113

Funes-Cravioto F, Kolmodin-Hedman B, Lindsten J, Nordenskjöld M, Zapata-
Gayon C, Lambert B, Norberg E, and Olin R (1977) Chromosome aberrations
and sister chromatid exchange in workers in chemical laboratories and
a rototyping factory and in children of women laboratory workers.
Lancet 2:322-325

- Galloway SM and Evans HJ (1975) Sister chromatid exchange in human chromosomes from normal individuals and patients with ataxia telangiectasia. *Cytogenet Cell Genet* 15:17-29
- Gebhart E, Lösing J, and Wopfner F (1980) Chromosome studies on lymphocytes of patients under cytostatic therapy. I. Conventional chromosome studies in cytostatic interval therapy. *Hum Genet* 55:53-63
- Gebhart E (1981) Sister chromatid exchange (SCE) and structural chromosome aberrations in mutagenicity testing. *Hum Genet* 58:235-254
- Goh K (1981) Sister chromatid exchange in the aging population. *J Med* 12:195-198
- Haglund U, Lundberg I, and Zech L (1980) Chromosome aberrations and sister chromatid exchanges in Swedish paint industry workers. *Scand J Work Environ Health* 6:291-298
- Hollander DH, Tockman MS, Liang YW, Borgaonkar DA, and Frost JK (1978) Sister chromatid exchanges in the peripheral blood of cigarette smokers and in lung cancer patients; and the effect of chemotherapy. *Hum Genet* 44:165-171
- Hopkin JM and Evans HJ (1979) Cigarette smoke condensates damage DNA in human lymphocytes. *Nature* 279:241-242
- Husgafvel-Pursiainen K, Mäki-Paakkanen J, Norppa H, and Sorsa M (1980)

Smoking and sister chromatid exchange. *Hereditas* 92:247-250

Husum B and Wulf HC (1980) Sister chromatid exchanges in lymphocytes in operating room personnel. *Acta Anaesthesiol Scand* 24:22

Husum B, Wulf HC, and Niebuhr E (1982) Increased sister chromatid exchange frequency in lymphocytes in healthy cigarette smokers. *Hereditas* 96:85-88

Högstedt B, Hedner K, Mark-Vendel E, Mitelman F, Schütz A, and Skerfving S (1979) Increased frequency of chromosome aberrations in workers exposed to styrene. *Scand J Work Environ Health* 5:333-335

Ikeda M, Koizumi A, Watanabe T, Endo A, and Sato K (1980) Cytogenetic and cytokinetic investigations on lymphocytes from workers occupationally exposed to tetrachloroethylene. *Toxicology Letters* 5:251-256

Ikushima T and Wolff S (1974) Sister chromatid exchanges induced by light flashes to 5-Bromodeoxyuridine- and 5-Iododeoxyuridine substituted chinese hamster chromosomes. *Exp Cell Res* 87:15-19

Kato H and Shimada H (1975) Sister chromatid exchanges induced by mitomycin C: a new method of detecting DNA damage at chromosomal level. *Mutat Res* 28:459-464

Kato H and Stich HF (1976) Sister chromatid exchanges in ageing and repair-deficient human fibroblasts. *Nature* 260:447-448

- Kato H (1977) Spontaneous and induced sister chromatid exchanges as revealed by the BrdU-labelling method. *Int Rev Cytol* 49:55-97
- Kato H and Sandberg AA (1977) The effect of sera on sister chromatid exchanges in vitro. *Exp Cell Res* 109:445-448
- Kriek E (1974) Carcinogenesis by aromatic amines. *Biochimica Biophysica Acta* 355:177-203
- KucEROVÁ M, PolÍvkova Z, and BátorA J (1979) Comparative evaluation of the frequency of chromosomal aberrations and the SCE numbers in peripheral lymphocytes of workers occupationally exposed to vinyl chloride monomer. *Mutat Res* 67:97-100
- Kurvink K, Bloomfield CD, Keenan KM, Levitt S, and Cervenka J (1978) Sister chromatid exchange in lymphocytes from patients with malignant lymphoma. *Hum Genet* 44:137-144
- Lambert B, Hansson K, Lindsten J, Sten M, and Werelius B (1976) Bromodeoxyuridine-induced sister chromatid exchanges in human lymphocytes. *Hereditas* 83:163-174
- Lambert B, Lindblad A, Nordenskjöld M, and Werelius B (1978) Increased frequency of sister chromatid exchanges in cigarette smokers. *Hereditas* 88:147-149

- Lambert B, Lindblad A, Holmberg K, and Francesconi D (1982) The use of sister chromatid exchange to monitor human populations for exposure to toxicologically harmful agents. In: Wolff S (ed) Sister chromatid exchange. John Wiley and sons, New York, pp 149-182
- Latt SA (1973) Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc Natl Acad Sci (USA)* 70:3395-3399
- Latt SA (1974) Sister chromatid exchange, indices of human chromosome damage and repair: detection by fluorescence and induction by mitomycin C. *Proc Natl Acad Sci (USA)* 71:3162-3166
- Latt SA, Stetten G, Juergens LA, Buchanan GR, and Gerald PS (1975) Induction by alkylating agents of sister chromatid exchanges and chromatid breaks in Fanconi's anemia. *Proc Natl Acad Sci (USA)* 72:4066-4070
- Latt SA and Juergens LA (1977) In: Hook EK and Porter IH (ed) Population cytogenetics: Studies in humans. Academic Press, New York, pp 217-236
- Lieberman MW, Baney RN, Lee RE, Sell S, and Farber E (1971) Studies on DNA repair in human lymphocytes treated with proximate carcinogens and alkylating agents. *Cancer Res* 31:1297-1306
- Lieberman MW, Sell S, and Farber E (1971) Deoxyribonucleoside incorporation and the role of hydroxyurea in a model lymphocyte system for studying

DNA repair in carcinogenesis. *Cancer Res* 31:1307-1312

Lindahl T and Nyberg B (1972) Rate of depurination of native deoxyribonucleic acid. *Biochemistry* 11:3610-3618

Lindblad A and Lambert B (1981) Relation between sister chromatid exchange, cell proliferation and proportion of B and T cells in human lymphocyte cultures. *Hum Genet* 57:31-34

McCann J and Ames BN (1976) Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals: Discussion. *Proc Natl Acad Sci (USA)* 73:950-954

McRae WD, McKinnon EA, and Stich HF (1979) The fate of UV-induced lesions affecting SCEs, chromosome aberrations and survival of CHO cells arrested by deprivation of arginine. *Chromosoma* 72:15-22

Monticone RE and Schneider EL (1979) Induction of sister chromatid exchanges in human cells by fluorescent light. *Mutat Res* 59:215-221

Morgan WF and Crossen PE (1981) Factors influencing sister chromatid exchange rate in cultured human lymphocytes. *Mutat Res* 81:395-402

Mäki-Paakkanen J, Sorsa M, and Vainio H (1980) Chromosome aberrations and SCE in lead exposed workers. In: "29. Nordiske Yrkeshygieniske Möte i Norge, 3-5 November, 1980". Oslo: Yrkeshygienisk institutt, p 30

- Mäki-Paakkanen J, Husgafvel-Pursiainen K, Kalliomäki P-L, Tuominen J, and Sorsa M (1980) Toluene-exposed workers and chromosome aberrations. *J Toxicol Environ Health* 6:775-781
- Norppa H, Sorsa M, Vainio H, Gröhn P, Heinonen E, Holsti L, and Nordman E (1980) Increased sister chromatid exchange frequencies in lymphocytes of nurses handling cytostatic drugs. *Scand J Work Environ Health* 6:299-301
- Obe G and Herha J (1978) Chromosomal aberrations in heavy smokers. *Hum Genet* 41:259-263
- Pedersen C, Oláh E, and Merrild U (1979) Sister chromatid exchanges in cultured peripheral lymphocytes from twins. *Hum Genet* 52:281-294
- Perry P and Wolff S (1974) New Giemsa method for the differential staining of sister chromatids. *Nature* 251:156-158
- Perry P and Evans HJ (1975) Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* 258:121-125
- Raposa T (1978) Sister chromatid exchange studies for monitoring DNA damage and repair capacity after cytostatics in vitro and in lymphocytes of leukaemic patients under cytostatic therapy. *Mutat Res* 57:241-251
- Sasaki MS, Tonomura A, and Matsubara S (1979) Chromosome constitution and its bearing on the chromosomal radiosensitivity in man. *Mutat Res* 10:617-633

Schmidt MA and Sanger WG (1981) Sister chromatid exchange in aged human lymphocytes

A brief note. Mechanisms of Ageing and Development 16:67-70

Schneider EL and Gilman B (1979) Sister chromatid exchanges and aging. III.

The effect of donor age on mutagen-induced sister chromatid exchange in human diploid fibroblasts. Hum Genet 46:57-63

Speit G (1980) Effects of temperature on sister chromatid exchanges. Hum

Genet 55:333-336

Taylor JH, Woods PS, and Hughes WL (1957) The organization and duplication of

chromosomes as revealed by autoradiographic studies using tritium-labelled thymidine. Biochemistry 43:122-127

Tough IM, Smith PG, Court Brown WM, and Harnden DG (1970) Chromosome studies

on workers exposed to atmospheric benzene. The possible influence of age. Europ J Cancer 6:49-55

Waksvik H, Magnus P, and Berg K (1981) Effects of age, sex and genes on sister

chromatid exchange. Clin Genet 20:449-454

Waksvik H, Klepp O, and Brögger A (1981) Chromosome analyses of nurses

handling cytostatic agents. Cancer Treat Rep 65:607

Waksvik H and Boysen M (1982) Cytogenetic analyses of lymphocytes from nickel

refinery workers. Mutat Res 103:185

Weinberg R, Avet LM, and Gardner MJ (1976) Estimates of the heritability of serum lipoprotein and lipid concentrations. Clin Genet 9:588-592

Watanabe T, Endo A, Kato Y, Shima S, Watanabe T, and Ikeda M (1980) Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers. Int Arch Occup Environ Health 46:31-41

Wolff S and Perry P (1974) Differential Giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. Chromosoma 48:341-353

Wolff S (1977) Sister chromatid exchange. Ann Rev Genet 11:183-201

Wolff S (1982) Chromosome aberrations, sister chromatid exchanges, and the lesions that produce them. In: Wolff S (ed) Sister chromatid exchange. John Wiley and sons, New York, pp 41-57

Relationship between sister chromatid exchanges and structural chromosome aberrations in lymphocytes of 100 individuals

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HEDNER, K., HÖGSTEDT, B., KOLNIG, A.-M., MARK-VENDEL, E., STRÖMBECK, B and MITELMAN, F. 1982. Relationship between sister chromatid exchanges and structural chromosome aberrations in lymphocytes of 100 individuals. — *Hereditas* 97: 237–245. Lund, Sweden. ISSN 0018-0661. Received April 8, 1982

Sister chromatid exchanges and structural chromosome aberrations, i.e. chromatid and isochromatid gaps, chromatid breaks and interchanges, acentric fragments, pericentric inversions, marker chromosomes, rings and dicentric chromosomes, were analyzed in peripheral blood lymphocytes of 100 individuals. The subjects belonged to six different groups: 22 factory workers occupationally exposed to ethylene oxide; 17 factory workers occupationally exposed to epoxy resins; 13 factory workers occupationally exposed to organic solvents, mainly toluene; 7 bus drivers; 6 chronic alcoholics; and 35 factory workers with no occupational exposure to any known genotoxicant. In the total material there was a highly significant correlation between the number of each structural aberration type recorded and the number of cells with such aberrations. There was no correlation between sister chromatid exchange frequency and the frequency of the different types of structural chromosome aberrations, neither in the different exposure groups, nor among unexposed individuals.

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Analysis of structural chromosome aberrations in peripheral blood lymphocytes is the only method which so far is practically useful in detecting genetic damage directly in man. As a consequence, considerable use is being made of this technique as a means of monitoring people exposed to potential mutagenic and/or carcinogenic agents. However, only gross genetic damage is detectable by this method, and the absence of chromosome aberrations does not exclude other types of DNA damage caused by environmental genotoxicants. The recent introduction of techniques for the detection of sister chromatid exchanges (SCE) has attracted much interest as a potentially more sensitive and rapid indicator of DNA damage than conventional chromosome aberrations. These exchange events can be readily observed in cells that have gone through two cell-cycles in the presence of 5-bromodeoxyuridine (BrdU) and have been stained with a fluorescent dye and/or Giemsa to give differential staining of the sister chromatids (LATT 1973; PERRY and WOLFF 1974). SCE involve equal symmetrical exchange at one locus between sister chromatids and thus do not result in any alteration of overall chromosome morphology; thus previously undetectable damage to the genetic material

might in this way be revealed. The biological significance of SCE is, however, to a large extent unknown and there are strong indications that SCE and structural chromosome aberrations arise by partly different mechanisms and reflect different kinds of primary DNA damage (see Discussion). Furthermore, very little information is available concerning the relationship between SCE and structural chromosome aberrations in reference populations and/or after low level chronic *in vivo* exposure to potentially mutagenic and/or carcinogenic agents.

This paper reports a study of the relationship between structural chromosome aberrations and SCE in peripheral lymphocytes of 100 individuals.

Material and methods

100 individuals (69 males and 31 females) were studied. The subjects belonged to six different exposure groups:

Cases No. 1–22: 22 healthy factory workers, 8 males and 14 females, aged 19–55 years, occu-

pationally exposed to ethylene oxide, from 0.5 to 8 years. Their working conditions have been described in detail elsewhere (HÖGSTEDT et al. 1983).

Cases No. 23–39: 17 healthy factory workers, 13 males and 4 females, aged 18–65 years, occupationally exposed to epoxy resins, from 3 to 16 years. Their working conditions have been described in detail elsewhere (MITELMAN et al. 1980).

Cases No. 40–52: 13 healthy factory workers, all males, aged 18–56 years, occupationally exposed to a variety of organic solvents, mainly toluene. They were working with glue production and had been exposed from 9 months to 12 years.

Cases No. 53–59: 7 healthy bus drivers, 6 males and 1 female, aged 22–44 years, who had been full time bus drivers for 3–21 years.

Cases No. 60–65: 6 individuals suffering from advanced chronic alcoholism, all males aged 32–51 years. The analysis was made on admission to hospital after about 2 weeks of excessive drinking. The concentration of ethanol in the blood at sampling was 79–126 mmol/l.

Cases No. 66–100: 35 healthy factory workers, 23 males and 12 females, aged 20–59 years, controls to Cases 1–39, matched for sex and age. They were workers in the same factories as Cases 1–39, but had no occupational exposure to any known genotoxicant (for details see MITELMAN et al. 1980; HÖGSTEDT et al. 1983).

All individuals were interviewed concerning recent virus infections, drug intake, exposure to ionizing radiation and smoking habits.

Structural chromosome aberrations were analyzed in peripheral blood lymphocytes according to the following microculture technique: 10 drops of whole blood were incubated at 37°C with 0.27 ml phytohemagglutinin (PHA) in 10 ml medium (80 % McCoy's 5A medium and 20 % fetal calf serum) for 72 h. Colchicin (0.1 µg/ml) was added 1 h before harvest. Hypotonic treatment was carried out with 0.075 mol/l KCl for 10–15 min in room temperature, and the cells were fixed 4 times in methanol: acetic acid 3:1. Fixed cells were dropped on dry slides, stained in 2 % Giemsa (Merck) for 5 min, rinsed and mounted.

All preparations were coded and analyzed with-

out any knowledge of the exposure data. All aberrations found were judged also by another observer, and agreement between the two was required before an aberration was accepted; metaphases with a questionable aberration were never rejected. 200 metaphases were analyzed from each individual and the aberrations were recorded according to the classification system recommended by ISCN (1978). The chromosome aberrations were referred to either as gaps (chromatid and isochromatid gaps), breaks (chromatid breaks and acentric fragments), and exchange type aberrations (chromatid interchanges, ring chromosomes, dicentric chromosomes, pericentric inversions and marker chromosomes).

Sister chromatid exchanges (SCE) were analyzed in peripheral lymphocytes from the same blood sample as used for analysis of structural chromosome aberrations. The outline of the procedure, essentially according to WOLFF and PERRY (1974), is as follows: 15 drops of whole blood were cultured for 72 h at 37°C in the dark with 0.27 ml PHA in 10 ml medium of the same composition as used for conventional chromosome analysis (see above). BrdU was added to each bottle to make a final concentration of 100 µmol/l medium and was present during the entire culture period. Colchicin (0.1 µg/ml) was added 1 h before harvest. Hypotonic treatment was carried out in the dark with 0.075 mol/l KCl for 20 min, and the cells were fixed in 3 changes of methanol: acetic acid, 3:1. Fixed cells were dropped on clean, dry slides and the slides were kept in the dark at room temperature for at least 3 days. Slides were stained in 5 µg/ml 33258 Hoechst in distilled water for 10 min, rinsed and placed in a lying position in newly made phosphate buffer, pH 7 (0.2 mol/l tri-Na-citrate: 0.2 mol/l NaH₂PO₄; 0.2 mol/l Na₂HPO₄, 1:2:2). UV-irradiation (30 W) was carried out for 15 h; the distance from slide to lamp was 60 cm. Slides were stained with 2 % Giemsa (Merck) for 5–7 min, rinsed and mounted.

20 metaphases with 46 chromosomes were analyzed from each individual on coded slides by one observer. Terminal SCEs were recorded as a single SCE and interstitial SCEs as two exchanges. Centromeric exchanges were included whenever no clear 180° twist could be observed. The average number of SCEs based on the 20 cells was used for further calculations of group mean values.

When analyzing the association between the effect parameters, the Pearson correlation coefficient

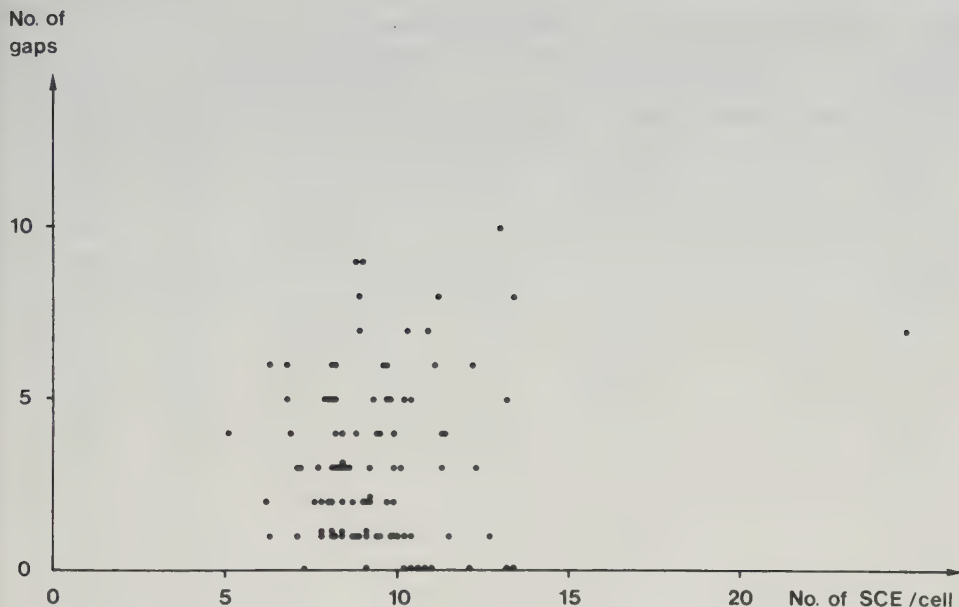


Fig. 1. Association between mean number of SCE per cell and number of gaps/200 cells in 100 individuals.

cient was used. Statistical significance denotes $P < 0.05$. All tests were two-sided.

Results

Age, sex and individual values for all the cytogenetic parameters studied are presented in Table 1. In the table, gaps include both chromatid and isochromatid gaps, breaks include chromatid breaks and acentric fragments, and exchanges include chromatid interchanges, pericentric inversions, ring chromosomes, dicentric chromosomes and marker chromosomes.

There was a highly significant correspondence between the number of gaps and the number of cells with gaps ($r = 0.9952$, $P < 0.001$), number of breaks and number of cells with breaks ($r = 0.9835$, $P < 0.001$), number of exchanges and number of cells with exchanges ($r = 0.9978$, $P < 0.001$) as well as between the total number of aberrations and the total number of cells with aberrations ($r = 0.9847$, $P < 0.001$). In the following, only the number of gaps, breaks and exchange type aberrations are presented.

Fig. 1–4 show the relationships between SCE frequency and number of gaps, breaks, exchanges, and total number of structural chromosome aberrations. There was no significant correlation between SCE frequency and any of the different types of structural chromosome aberrations recorded (gaps, $r = 0.0142$; breaks, $r = 0.1887$; exchanges, $r = 0.0877$; total No. of aberrations, $r = 0.1624$). The lack of correlation was evident both within each of the different exposure groups as well as among the control individuals.

Discussion

There is a considerable literature on in vivo and in vitro studies of numerical and/or structural chromosome aberrations in somatic cells of animals and man. Major limitations for this assay system are the high degree of skill required on the part of the scorer, and inter-scorer and inter-laboratory variations in scoring criteria. Also, the method is laborious and time-consuming, and the capacity for extensive analyses in existing laboratories is limited.

Table 1. Age, sex and frequencies of the cytogenetic parameters studied in 100 individuals

Case No.	Age	Sex	Number of aberrations				Number of cells with aberrations				Mean No. of SCE/cell
			Gaps	Breaks	Exchanges	Total	Gaps	Breaks	Exchanges	Total	
1	34	F	6	3	1	10	6	3	1	10	9.7
2	53	F	4	9	1	14	4	9	1	14	9.9
3	19	F	10	11	1	22	9	10	1	19	13.0
4	20	F	4	3	0	7	4	3	0	7	9.4
5	53	F	8	8	1	17	8	8	1	15	13.4
6	51	F	1	8	4	13	1	5	3	9	9.8
7	54	F	8	6	1	15	8	5	1	13	11.2
8	19	F	1	3	0	4	1	3	0	4	8.9
9	51	F	2	3	3	8	2	2	3	7	9.2
10	29	F	7	10	2	19	6	9	2	16	10.3
11	37	F	9	9	1	19	9	8	1	17	9.0
12	24	M	5	7	0	12	5	7	0	12	6.8
13	34	F	3	14	0	17	3	11	0	14	11.3
14	54	F	7	6	1	14	7	6	1	13	10.9
15	39	F	5	14	0	19	4	11	0	15	13.2
16	25	M	3	2	0	5	3	2	0	5	9.9
17	26	M	1	1	0	2	1	1	0	2	8.1
18	20	M	0	2	0	2	0	2	0	2	11.0
19	55	M	7	5	0	12	7	5	0	12	24.9
20	23	M	6	2	0	8	6	2	0	8	8.2
21	42	M	5	2	0	7	5	2	0	7	10.4
22	49	M	2	1	0	3	2	1	0	3	8.4
23	21	M	1	1	1	3	1	1	1	3	8.2
24	25	F	0	2	0	2	0	2	0	2	13.4
25	32	M	1	2	0	3	1	2	0	3	8.7
26	26	M	4	3	0	7	3	3	0	6	11.3
27	18	F	1	3	0	4	1	3	0	4	12.7
28	41	M	1	2	0	3	1	2	0	3	10.4
29	58	F	3	2	0	5	3	2	0	5	7.1
30	35	M	1	1	0	2	1	1	0	2	9.1
31	59	M	6	2	0	8	6	2	0	8	6.3
32	55	M	5	3	0	8	4	3	0	7	9.8
33	65	M	2	3	1	6	2	3	1	6	6.2
34	34	M	2	2	0	4	2	2	0	4	9.9
35	35	F	3	3	1	7	2	3	1	6	12.2
36	29	M	1	2	0	3	1	2	0	3	6.3
37	53	M	5	3	0	8	4	2	0	6	9.7
38	29	M	4	1	0	5	4	1	0	5	5.1
39	50	M	3	5	1	9	3	5	1	8	8.2
40	47	M	1	7	0	8	1	7	0	8	9.1
41	18	M	2	0	1	3	2	0	1	3	8.7
42	32	M	5	6	0	11	5	5	0	9	7.9
43	44	M	2	7	0	9	2	7	0	9	7.6
44	18	M	4	4	1	9	4	4	1	9	8.8
45	36	M	3	0	0	3	3	0	0	3	9.2
46	56	M	9	5	0	14	8	5	0	13	8.8
47	18	M	5	2	0	7	5	2	0	7	9.3
48	30	M	0	3	0	3	0	2	0	2	9.1
49	28	M	1	2	0	3	1	2	0	2	7.8
50	18	M	5	2	1	8	5	2	1	8	8.2

Table 1. (cont.)

Case No.	Age	Sex	Number of aberrations				Number of cells with aberrations				Mean No. of SCE/cell
			Gaps	Breaks	Exchanges	Total	Gaps	Breaks	Exchanges	Total	
51	32	M	3	3	0	6	3	3	0	5	7.7
52	37	M	7	6	0	13	6	6	0	12	8.9
53	46	M	4	3	0	7	4	3	0	7	9.5
54	48	M	6	5	1	12	6	4	1	9	6.8
55	44	M	1	2	1	4	1	2	1	4	9.5
56	25	F	4	1	0	5	4	1	0	5	8.2
57	24	M	4	7	2	13	4	7	2	13	8.4
58	31	M	1	6	1	8	1	6	1	8	8.8
59	36	M	5	9	1	15	5	7	1	12	8.1
60	49	M	5	2	2	9	5	2	2	9	10.2
61	51	M	4	4	1	9	4	3	1	8	11.4
62	41	M	2	1	1	4	2	1	1	4	9.7
63	50	M	0	3	1	4	0	3	1	4	10.6
64	32	M	0	2	1	3	0	1	1	2	13.2
65	38	M	6	3	1	10	6	3	1	10	9.6
66	43	F	6	9	5	20	5	8	4	16	12.2
67	50	F	0	4	0	4	0	4	0	4	12.1
68	23	F	6	4	0	10	6	3	0	8	11.1
69	58	F	8	5	3	16	6	5	3	14	8.9
70	48	F	6	3	1	10	6	3	1	8	8.1
71	31	F	3	4	0	7	3	4	0	7	8.6
72	21	F	1	4	1	6	1	4	1	4	11.5
73	50	F	2	10	4	16	2	9	3	14	9.1
74	53	F	0	2	2	4	0	2	2	4	10.4
75	53	F	2	7	0	9	2	7	0	9	9.2
76	41	M	1	1	1	3	1	1	1	3	10.0
77	24	M	2	4	0	6	2	3	0	5	8.0
78	32	M	1	3	1	5	1	2	1	4	8.4
79	21	M	2	3	1	6	2	2	1	5	8.1
80	23	M	0	3	0	3	0	3	0	3	10.2
81	21	M	2	1	0	3	2	1	0	3	7.8
82	20	M	3	6	0	9	3	6	0	9	8.4
83	44	M	1	1	0	2	1	1	0	2	7.8
84	38	M	3	1	0	4	3	1	0	4	8.5
85	21	F	0	1	0	1	0	1	0	1	10.8
86	27	M	1	2	0	3	1	2	0	3	10.2
87	33	M	3	4	0	7	3	3	0	5	10.1
88	28	M	1	1	0	2	1	1	0	2	9.9
89	41	M	1	4	0	5	1	3	0	4	9.4
90	49	F	3	2	0	5	3	2	0	5	7.2
91	56	M	3	5	0	8	3	4	0	7	8.3
92	35	M	3	1	0	4	3	1	0	4	8.1
93	59	M	1	2	0	3	1	2	0	3	8.1
94	56	M	4	8	1	13	4	6	1	10	6.9
95	29	M	3	5	1	9	3	5	1	9	8.4
96	50	M	5	4	0	9	4	4	0	8	8.0
97	29	M	1	1	1	3	1	1	1	3	7.1
98	28	M	1	11	0	12	1	9	0	10	8.4
99	45	M	0	8	0	8	0	8	0	8	7.3
100	38	M	2	6	0	8	2	6	0	8	9.0

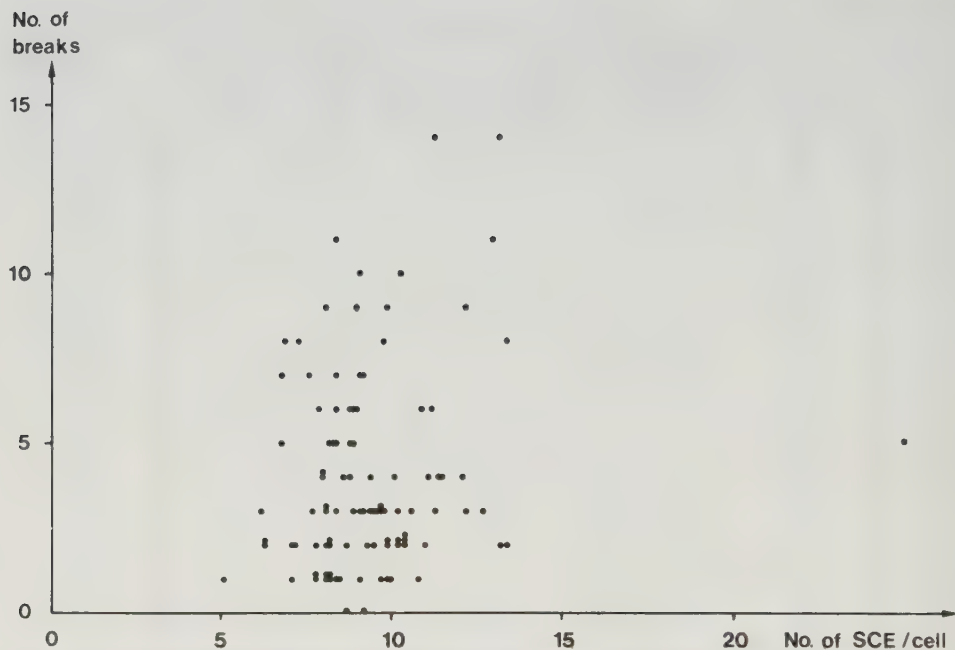


Fig. 2. Association between mean number of SCE per cell and number of breaks/200 cells in 100 individuals.

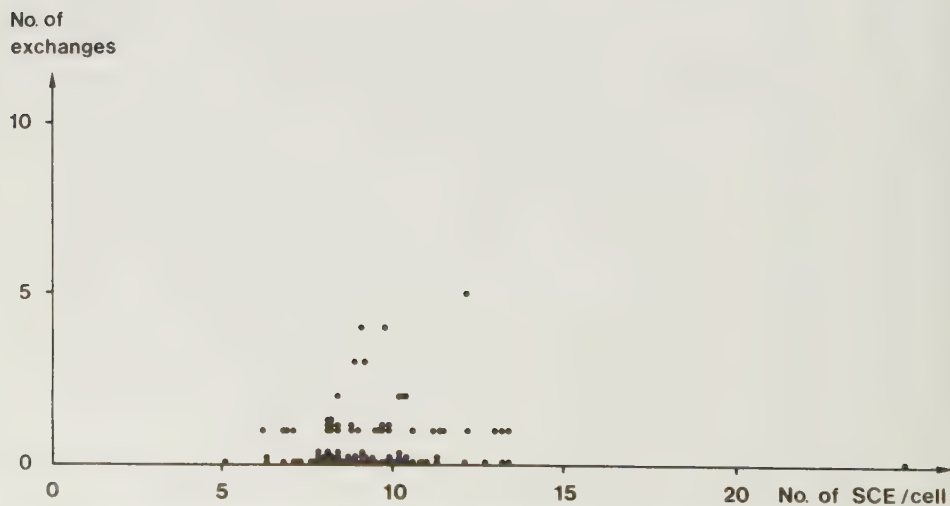


Fig. 3. Association between mean number of SCE per cell and number of exchange type aberrations/200 cells in 100 individuals.

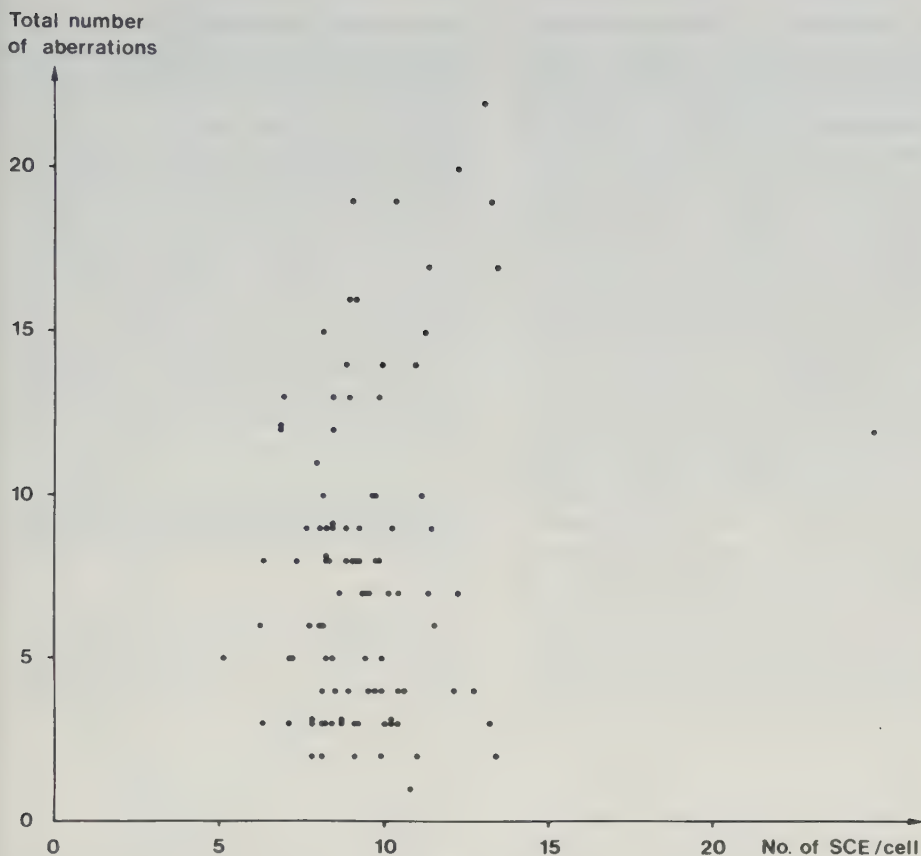


Fig. 4. Association between mean number of SCE per cell and total number of structural chromosome aberrations/200 cells in 100 individuals.

Since the development of the BrdU-staining techniques for the morphologic differentiation of sister chromatids (LATT 1973; PERRY and WOLFF 1974), investigations of SCE frequency have become part of the test battery of genotoxicology screening systems as an easily observable and potentially more sensitive indicator of chemically induced chromosome damage than the traditional chromosome breakage studies (VOGEL and BAUKNECHT 1976; CARRANO et al. 1978). Significant increases in SCE have been induced in cultured cells by a number of chemicals at levels that produce little or no gross chromosome change (e.g., LATT 1974; PERRY and EVANS 1975; KATO and SHIMADA 1975; RUDIGER et al. 1976; WOLFF 1977; RAPOSA 1978; BRÖGGER 1982). However, it appears that the

mechanisms leading to the formation of SCE are heterogeneous and fundamentally different from those that cause structural chromosome aberrations (GEBHART 1981). For example, agents that produce severe chromosome breakage, such as X-rays, have little or no effect on SCE frequency (PERRY and EVANS 1975). Also, among patients with hereditary chromosome instability disorders, such as ataxia telangiectasia, Bloom's syndrome and Fanconi's anemia, characterized by a markedly increased frequency of spontaneous chromosome aberrations, only patients with Bloom's syndrome display an increase in SCE (CHAGANTI et al. 1974) while patients with ataxia telangiectasia and Fanconi's anemia have a normal frequency (GALLOWAY and EVANS 1975; LATT et al.

1975). Furthermore, the demonstration of SCE is influenced by a variety of technical factors. Although it can be presumed that SCEs occur spontaneously, a considerable proportion of them is induced by BrdU itself (WOLFF and PERRY 1974; GALLOWAY and EVANS 1975; LAMBERT et al. 1976), and the response to BrdU may be complex (LATT and JUERGENS 1977). Other factors also exert influence, for instance the composition of the medium, especially the serum (KATO and SANDBERG 1977), the staining method (MORGAN and CROSSEN 1981), light (IKUSHIMA and WOLFF 1974; MONTICONE and SCHNEIDER 1979), temperature (SPEIT 1980), and the rate of cell proliferation (LINDBLAD and LAMBERT 1981). Obviously fundamentally different lesions can result in SCE formation, as SCEs are induced and/or influenced by a variety of chemical and physical agents producing different types of DNA lesions. Also, genetic factors may be of importance. Although studies of twins indicate that genetic variance does not have any major influence on the variation of spontaneous SCE rates (PEDERSEN et al. 1979; WAKSVIK et al. 1981), other results (SASAKI et al. 1970; COUNTRYMAN and HEDDLE 1976) clearly suggest genetically determined increased susceptibility and/or decreased resistance to chromosome-damaging effects of environmental agents. In this respect, the effects on different cytogenetic parameters may be entirely different.

In the present study, the frequencies of SCE and chromosome aberrations were compared in peripheral blood lymphocytes from 100 individuals—65 persons belonging to five different exposure groups, and 35 persons without exposure to any known genotoxicant. We found no correlation between the frequency of SCE and any of the various types of chromosome aberrations studied in the total material. This lack of correlation was evident within the normal range of chromosome variation, as well as in individuals exposed to ethylene oxide—the exposure group with the highest level of structural chromosome aberrations. In this context it may be of interest to mention that this group of individuals has previously been shown to have an increased frequency of chromosome aberrations, but not of SCE, when compared to an appropriate control group (HOGSTEDT et al. 1983).

Very little information is available as regards the relationship between SCE and chromosome aberrations in vivo in other groups of individuals, especially in reference populations and after low level in vivo exposure, and the lack of correlation

between these two parameters in our study is therefore difficult to evaluate. In a recent review (BRÖGGER 1982) 21 studies of exposed groups of people were compared, from whom samples were analyzed both for chromosome aberrations and SCE. It appears from this comparison that the SCE method generally is less effective in revealing cytogenetic damage than the analysis of chromosome aberrations. In fact, negative chromosome aberration analysis and a positive SCE test was not found in any instance. This is in clear contrast to reports from most in vitro studies, where SCE has been found to be the more sensitive method of revealing DNA damage. The picture might change as more chemicals are investigated, but based on the observations available, analysis of structural chromosome aberrations appears to be preferable in investigations of chronic in vivo exposure.

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Literature cited

- BRÖGGER, A. 1982. Application of SCE to public health. — In *Sister Chromatid Exchange* (Ed. A. A. SANDBERG), *Alan Liss Inc., New York*, p. 655–673
- CARRANO, A. V., THOMPSON, L. H., LINDL, P. A. and MINKLER, J. L. 1978. Sister chromatid exchange as an indicator of mutagenesis. — *Nature* 271: 551–553
- CHAGANTI, R. S. K., SCHONBERG, S. and GERMAN, J. 1974. A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. — *Proc. Natl. Acad. Sci. (USA)* 71: 4508–4512
- COUNTRYMAN, P. and HEDDLE, J. 1976. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. — *Mutat. Res.* 41: 321–332
- GALLOWAY, S. M. and EVANS, H. J. 1975. Sister chromatid exchange in human chromosomes from normal individuals and patients with ataxia telangiectasia. — *Cytogenet. Cell. Genet.* 15: 17–29
- GEBHART, E., 1981. Sister chromatid exchange (SCE) and structural chromosome aberrations in mutagenicity testing. — *Hum. Genet.* 58: 235–254
- HOGSTEDT, B., GULLBERG, B., HEDNER, K., KOLNIG, A.-M., MITELMAN, F., SKERFVING, S. and WIDEGREN, B. 1983. Chromosome aberrations and micronuclei in bone marrow cells and peripheral blood lymphocytes in humans exposed to ethylene oxide. — *Hereditas* 98: 000–000
- IKUSHIMA, T. and WOLFF, S. 1974. Sister chromatid exchanges induced by light flashes to 5-bromodeoxyuridine and 5-iododeoxyuridine substituted Chinese hamster chromosomes. — *Exp. Cell Res.* 87: 15–19
- ISCN 1978. An International System for Human Cytogenetic Nomenclature. — *Cytogenet. Cell Genet.* 21: 309–404
- KATO, H. and SANDBERG, A. A. 1977. The effect of sera on sister chromatid exchanges in vitro. — *Exp. Cell Res.* 109: 445–448

- KATO, H and SHIMADA, H. 1975. Sister chromatid exchanges induced by Mitomycin C: a new method of detecting DNA damage at chromosomal level. — *Mutat. Res.* 28: 459–464
- LAMBERT, B., HANSSON, K., LINDSTEN, J., STEN, M. and WERELIUS, B. 1976. Bromodeoxyuridine-induced sister chromatid exchanges in human lymphocytes. — *Hereditas* 83: 163–174
- LATT, S. 1973. Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. — *Proc. Natl. Acad. Sci. (USA)* 70: 3395–3399
- LATT, S. 1974. Sister chromatid exchanges, indices of human chromosome damage and repair: detection by fluorescence and induction by Mitomycin C. — *Proc. Natl. Acad. Sci. (USA)* 71: 3162–3166
- LATT, S. A. and JUERGENS, L. A. 1977. Determinants of sister chromatid exchange frequencies in human chromosomes. — In *Population Cytogenetics: Studies in Humans* (Ed. E. B. HOOK and I. H. PORTER), Academic Press, New York, p. 217–236
- LATT, S. A., STETTEN, G., JUERGENS, L. A., BUCHANAN, G. R. and GERALD, P. S. 1975. Induction by alkylating agents of sister chromatid exchanges and chromatid breaks in Fanconi's anemia. — *Proc. Natl. Acad. Sci. (USA)* 72: 4066–4070
- LINDBLAD, A. and LAMBERT, B. 1981. Relation between sister chromatid exchange, cell proliferation and proportion of B and T cells in human lymphocyte cultures. — *Hum. Genet.* 57: 31–34
- MITELMAN, F., FREGERT, S., HEDNER, K. and HILLBERTZ-NILSSON, K. 1980. Occupational exposure to epoxy resins has no cytogenetic effect. — *Mutat. Res.* 77: 345–348
- MONTICONE, R. and SCHNEIDER, E. 1979. Induction of sister chromatid exchanges in human cells by fluorescent light. — *Mutat. Res.* 59: 215–221
- MORGAN, W. F. and CROSSEN, P. E. 1981. Factors influencing sister chromatid exchange rate in cultured human lymphocytes. — *Mutat. Res.* 81: 395–402
- PEDERSEN, C., OLAH, E. and MERRILD, U. 1979. Sister chromatid exchanges in cultured peripheral lymphocytes from twins. — *Hum. Genet.* 52: 281–294
- PERRY, P. and EVANS, H. J. 1975. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. — *Nature* 258: 121–125
- PERRY, P. and WOLFF, S. 1974. New Giemsa method for the differential staining of sister chromatids. — *Nature* 251: 156–158
- RAPOSA, T. 1978. Sister chromatid exchange studies for monitoring DNA damage and repair capacity after cytostatics in vitro and in lymphocytes of leukemic patients under cytostatic therapy. — *Mutat. Res.* 57: 241–251
- RUDIGER, H. W., KOHL, F., MANGELS, W., WICHERT, P. von, BARTRAM, C. R., WÖHLER, W. and PASSARGE, E. 1976. Benzpyrene induces sister chromatid exchanges in cultured human lymphocytes. — *Nature* 262: 290–292
- SASAKI, M. S., TONOMURA, A. and MATSUBARA, S. 1970. Chromosome constitution and its bearing on the chromosomal radiosensitivity in man. — *Mutat. Res.* 10: 617–633
- SPEIT, G. 1980. Effects of temperature on sister chromatid exchanges. — *Hum. Genet.* 55: 333–336
- VOGEL, W. and BAUKNECHT, T. 1976. Differential chromatid staining by in vivo treatment as a mutagenicity test system. — *Nature* 260: 448–449
- WAKSVIK, H., MAGNUS, P. and BERG, K. 1981. Effects of age, sex and genes on sister chromatid exchange. — *Clin. Genet.* 20: 449–454
- WOLFF, S. 1977. Sister chromatid exchange. — *Ann. Rev. Genet.* 11: 183–201
- WOLFF, S. and PERRY, P. 1974. Different Giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. — *Chromosoma* 48: 341–353

OCCUPATIONAL EXPOSURE TO EPOXY RESINS HAS NO CYTOGENETIC EFFECT

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Summary

Cytogenetic evaluation was performed of peripheral lymphocytes from 18 workers currently exposed to epoxy resins of the type diglycidyl ether bisphenol-A (DGEBA). 9 workers were exposed to low molecular (average MW <900) and 9 to high molecular (average MW about 2000) epoxy resins. The results were compared with an equal number of control individuals matched for sex and age. There was no difference in chromosomal aberration frequency between the controls and the groups exposed to epoxy resin. Thus, this study gave no indication that exposure to epoxy resin produces visible damage to the chromosomes.

Epoxy resins are commonly used for protective coatings, and in glues and paints. Commercial epoxy resin of the bisphenol-A type (DGEBA) constitutes about 90% of all epoxy resins and is almost the only one used in the building industry. They are mixtures of different types of oligomer with molecular weights of 340, 624, 908, 1192, etc. [6]. A mutagenic and/or carcinogenic action of aromatic epoxy resins has long been suspected, but results so far obtained have been contradictory [1,7]. To our knowledge, no cytogenetic study has been performed of individuals exposed to epoxy resins. The current study was taken to examine the possible chromosome-damaging effect in lymphocytes from individuals occupationally exposed to low and high molecular epoxy resins.

Material and methods

4 groups of individuals were studied.

(1) 9 individuals (aged 30–66 years, median 45.4 years) occupationally

exposed to low molecular epoxy resins for 5–16 years (median 6.5 years). All workers were from the same factory where they glued metal and rubber. The epoxy resin was of the type diglycidyl ether bisphenol-A. The average MW was below 900 and the main oligomer was of MW 340. The hardener used was of aliphatic amine type. No reactive diluent had been used. Acetone was used to remove surplus glue. There was a well-developed protective programme. Textile gloves were used, but intermittent contamination of the skin could not be prevented.

(2) 9 controls matched for sex and age. The control workers carried out metal-work in the same factory but had not been occupationally exposed to epoxy compounds.

(3) 9 individuals (aged 18–59 years, median 30.1 years) occupationally exposed to high molecular epoxy resins for 3–10 years (median 7 years). The workers examined were from 3 factories where they used epoxy resin powder for electrostatic coating of metal. The epoxy resin was of the type diglycidyl ether bisphenol-A. The average molecular weight was about 2000. The epoxy powder contained dicyandiamide as hardener but no reactive diluent. Lead chromate was present in some batches. The spraying of powder was carried out by hand with pistols in ventilated boxes or automatically in closed boxes. Several times a week the boxes were rinsed by hand to remove surplus powder. The clothes and the skin of face and hands were more or less contaminated by the epoxy powder. During the hand-spraying and the rinsing-procedure nose and mouth were protected by a simple paper mask.

(4) 9 controls matched for sex and age. The control workers carried out metal-work but had not been occupationally exposed to epoxy compounds.

None of the individuals investigated was allergic to oligomer MW 340, which is the main sensitizer in epoxy resins [2].

The frequency of chromosomal aberrations was analyzed in peripheral lymphocytes cultured for 72 h by standard microculture technique. The chromosome preparations from all samples were randomized and scored under code by one observer. When difficulty was found in the interpretation of an aberration, the opinion of a second observer was sought, and agreement between the two observers was required for scoring the cells as normal or abnormal; such cells were not rejected; 200 metaphases were analyzed from each individual.

The frequency of sister-chromatid exchanges (SCE) in PHA-stimulated lymphocytes was studied with the Giemsa technique essentially according to Wolff and Perry [8]. BrDU (5-bromodeoxyuridine) was present in all cultures throughout the entire culture period at a final concentration of 0.1 mmoles/ml medium; cultures were harvested after 72.5 h. 20 cells with 46 chromosomes were examined in each individual.

Results

The pertinent results from this study are presented in Table 1, which summarizes the chromosome data from a total of 7200 cells, pooled for each group. Breaks + exchanges include chromatid breaks, chromatid interchanges, acentric fragments, ring chromosomes, dicentric chromosomes and pericentric inversions. Gaps represent both chromatid and isochromatid gaps. An aberration

TABLE 1

CHROMOSOMAL ABERRATIONS IN INDIVIDUALS EXPOSED TO EPOXY RESINS AND MATCHED CONTROLS

Group	Median age (yr)	Number of cells analyzed	Number of aberrations (%)			Number of cells with aberrations (%)			Number of SCE/cell
			Gaps	Breaks + exchanges	Total	Gaps	Breaks + exchanges	Total	
Epoxy — low mol.	45.4	1800	1.7	1.5	3.2	1.6	1.4	2.9	7.9
Controls	45.7	1800	1.3	1.8	3.1	1.2	1.6	2.9	7.8
Epoxy — high mol.	30.1	1800	0.7	0.9	1.7	0.7	0.9	1.6	10.1
Controls	33.2	1800	0.8	1.2	1.9	0.8	1.0	1.7	9.5
Total exposed	37.8	3600	1.2	1.2	2.4	1.1	1.2	2.3	9.0
Total controls	40.7	3600	1.1	1.6	2.6	1.1	1.9	2.9	8.7

tion was recorded as a break only when clear displacement occurred.

There was no difference between the controls and the groups exposed to epoxy resin. This was true for all types of aberration recorded, including the frequency of sister-chromatid exchanges.

Discussion

Almost 90% of commercial epoxy resins used are manufactured by condensation of epichlorohydrin with one or two molecules of bisphenol-A. The mutagenic action of epichlorohydrin has been reported for a wide range of organisms, including *Drosophila*, bacteria, *Neurospora*, mice and Chinese hamsters [5]. An increased frequency of chromosomal aberrations has been found in human cells exposed in vitro to epichlorohydrin [5] as well as in lymphocytes of workers occupationally exposed to epichlorohydrin [3,4]. Recently, a mutagenic action in the Ames test was reported for 3 DGEBA epoxy resin mixtures with an average molecular weight of 370, 950 and 1800 [1]. In another recent study the epoxy oligomer DGEBA with a molecular weight of 340, however, was not mutagenic in *S. typhimurium*. Neither were mixtures of epoxy oligomers of higher molecular weight mutagenic [7].

The present study did not give any indication that occupational exposure to low or high molecular epoxy resin produces visible chromosomal damage. These results should, however, be interpreted with caution. It is generally agreed that 48–52 h cultures will issue first-division cells, 72 h will give first, second and third divisions. The use of a 72-h fixation time for the assay of chromosomal aberrations may therefore result in a loss of cells with aberrations. It should also be mentioned that only G₀ cells are assayed in a peripheral blood investigation; cells in other stages of the cycle may be more sensitive. Another important factor may be the type of exposure. The subjects in our study who worked with low molecular weight resins were exposed by skin contact, but probably not by inhalation as the epoxy resin has a low vapour

pressure. The subjects who worked with powdered epoxy resin of high molecular weight were heavily exposed by skin contamination and probably also by inhalation. Thus, the bulk of exposure was to surface contamination, and it cannot be excluded that individuals exposed by inhalation receive higher doses which may raise the level of chromosomal aberrations above normal.

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References

- 1 Andersen, M., P. Kiel, H. Larsen and J. Maxild, Mutagenic action of aromatic epoxy resins, *Nature* (London), 276 (1978) 391—392.
- 2 Fregert, S., and A. Thorgeirsson, Patch testing with low molecular oligomers of epoxy resins in human, *Contact Dermatitis*, 3 (1977) 301—303.
- 3 Kucerova, M., V.S. Zhurkov, Z. Polivkova and J.E. Ivanove, Mutagenic effect of epichlorohydrin, II. Analysis of chromosomal aberrations in lymphocytes of persons occupationally exposed to epichlorohydrin, *Mutation Res.*, 48 (1977) 355—360.
- 4 Picciano, D., Cytogenetic investigation of occupational exposure to epichlorohydrin, *Mutation Res.*, 66 (1979) 169—173.
- 5 Šrám, R., M. Cerna and M. Kucerova, The genetic risk of epichlorohydrin as related to the occupational exposure, *Biol. Zentralbl.*, 95 (1976) 451—462.
- 6 Tanaka, Y., and A. Okada, Synthesis and characteristics of epoxides, in: C.A. May and Y. Tanaka (Eds.), *Epoxy Resins, Chemistry and Technology*, Marcel Dekker, New York, 1973, p. 9.
- 7 Wade, M.J., J.W. Moyer and C.H. Hine, Mutagenic action of a series of epoxides, *Mutation Res.*, 66 (1979) 367—371.
- 8 Wolff, S., and P. Perry, Differential Giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography, *Chromosoma*, 48 (1974) 341—352.

Chromosome aberrations and micronuclei in bone marrow cells and peripheral blood lymphocytes in humans exposed to ethylene oxide

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Twenty-eight individuals occupationally exposed to ethylene oxide have been compared with 20 controls regarding cytogenetic effects in peripheral blood lymphocytes and in bone marrow cells. The exposure levels of ethylene oxide were estimated and none of the individuals had, during the last 2.5 years, been exposed to workroom air concentrations exceeding 1 ppm. The exposed persons showed significantly increased levels of chromosome aberrations in lymphocytes (about 50 % increase of mean) and of micronuclei in erythroblasts and polychromatic erythrocytes (about 100 % and 300 % increase of mean, respectively) when compared with controls. However, sister chromatid exchanges and micronuclei in lymphocytes did not show any significant effect of exposure. A significant effect of smoking on micronuclei in erythroblasts and lymphocytes was found. There were several statistically significant, positive correlation coefficients among the different cytogenetic parameters.

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Ethylene oxide has alkylating properties that are due to the epoxide structure of the molecule. It reacts with DNA primarily at the N-7 position of guanine to form N-7-hydroxyethyl-guanine (BROOKES and LAWLEY 1961; EHRENBURG et al. 1974). It may also have affinity to other macromolecules (e.g., enzymes) in the body. Ethylene oxide has an inhibitory effect on the human DNA-repairing mechanism both in vitro and in vivo (PERO et al. 1981; PERO et al. 1982). It has a well documented ability to cause mutations in different test systems; in bacteria (PFEIFFER and DUNKELBERG 1980; RANNUG et al. 1976), in plants (EHRENBURG et al. 1956), in *Drosophila* (RAPOPORT 1948) and in mammals (APPELGREN et al. 1978; CONAN et al. 1979; EMBREE and HINE 1975).

Already in 1961, Ehrenburg and Hällström (KALLINGS 1967) obtained indications that persons highly exposed to ethylene oxide had an increased frequency of chromosomal aberrations in lymphocytes, but they investigated only a few metaphases in a small number of persons. The same results have recently been obtained by ABRAHAM (1980). Evidence has also been published indicating that ethylene oxide may cause haematological and

other types of malignancies in animals and man (DUNKELBERG 1979; HÖGSTEDT et al. 1979a, b).

The aims of the present study were to clarify whether ethylene oxide really causes chromosomal damage in man as measured by several cytogenetic methods, i.e. chromosomal aberrations, sister chromatid exchanges, and micronuclei in peripheral blood lymphocytes, and also chromosomal aberrations and micronuclei in bone marrow cells. The relation between levels of ethylene oxide in workroom air and the cytogenetic effects, and the association between the different cytogenetic parameters have also been studied.

Materials and methods

1. Interviews, physical examination and biological sampling

All subjects in this investigation, exposed as well as controls, were examined and interviewed by the same persons about diseases, drug intake, exposure to ionizing radiation or compounds with known or suspected mutagenic ability. Blood

samples from both the exposed workers and the controls from factory I were obtained on three consecutive days (October 1978) and from factory II five months later (March 1979) on two consecutive days. Bone marrow specimens of the workers in factory I were sampled in October 1978 and of the controls in May 1978.

2. Analysis of ethylene oxide air concentrations

Workroom air specimens were collected in whole-glass syringes (50 ml) during 30 min or 2 h by personal and stationary area sampling. The air samples were analyzed on a AID Portable Gas Chromatograph Model 514 equipped with a flame-ionization detector using a 2 m, 1/8" stainless steel column packed with Purepac Q, 100–120 mesh. The carrier gas was N₂, flow rate 20 ml/min; column temperature 150°C. The detection limit was 0.2 ppm but, in the measurements during 1975, levels below 1 ppm were unfortunately denoted in the protocol by a value of zero. Therefore, in Table 1, the exposure levels in some cases are given as a range, where the limits are calculated from the detection limits (1.0 and 0.2 ppm) and from zero ppm. Time-weighted averages (TWA) of exposure were calculated.

3. Working conditions and subjects studied

3.1 Factory I

Working conditions. — Medical equipment wrapped in plastic foil was treated in two sterilizers with a mixture of 50 % ethylene oxide and 50 % methyl formate. After treatment the sterilizers were air-flushed several times. They were opened for a period of 30 min, twice a day. The sterilizers were unloaded and the equipment placed in a ven-

tilated stockroom for 24 h, after which it was packed in paper boxes in a special workroom. The sterilizing personnel were exposed to ethylene oxide when loading and unloading the sterilizers (Table 1; momentary peak levels 52 ppm), which in all took 1–2 h a day. During the rest of the working day they were not exposed. One year before the study the sterilizing personnel began to wear breathing protective devices while the sterilizers were open. During the sterilization process ethylene oxide penetrates the plastic covers of the equipment. For several days afterwards it diffuses and thus pollutes the air of the stockroom (Table 1) and of the packingroom. A laboratory assistant worked in the stockroom for approximately 1 h a day. An attendant was exposed to ethylene oxide for a few hours a week while changing the gas cylinders. Between measurements in June and December 1975 the working routines were changed and consequently the levels decreased considerably (Table 1). No further changes were made until biological sampling in October 1978.

Exposed persons. — The exposed persons (Table 2) consisted of 18 individuals (17 females and 1 male) aged 19 to 55 (mean 37) years. They had been exposed to ethylene oxide for 0.5–8 (mean 3.2) years. Four were sterilizing and 12 were packing personnel, 1 was a laboratory worker, and 1 a male attendant.

Taking into consideration levels of ethylene oxide, working routines and use of protective devices it may be concluded that at the time of biological sampling in October 1978, all subjects were exposed to 8 h TWA levels below 1 ppm. Until three years earlier, the exposure levels were higher, though probably not exceeding 5 ppm. Five persons had had various X-ray examinations within one year before the investigation. Four persons took hormones as contraceptives or against

Table 1. Ethylene oxide levels (ppm, TWA) for different types of work operations

	Factory I				Factory II	
	June, 1975		December, 1975		May, 1978	
	Personal sampling	Area sampling	Personal sampling	Area sampling	Personal sampling	Area sampling
<i>Sterilization room</i>						
sterilizer open	29	28	7.6	5.0	2.4*	1.3*
sterilizer closed	8.9	6.4	1.3	0.6		
<i>Stockroom</i>	—	12	—	1.8	—	0.8
<i>Packing room</i>	4.5	4.4	0.3–1	0.1–1	0.1	—

* Sampling during total shifts

Table 2. Background data and result of cytogenetic analyses in persons exposed to ethylene oxide and controls (ND = not done)

No.*	Age	Sex	Smoking	Peripheral blood lymphocytes					Bone marrow cells		
				Cells with chromosomal aberrations			SCE (Mean No./ cell)	Micro- nuclei (%)	Chromo- somal aberra- tions (%)	Micronuclei in	
				Breaks (%)	Gaps (%)	Total (%)				Erythro- blasts (%)	Polychromatic erythrocytes (%)
1	58	F	no	2.5	1	3.5	9	10	0	2	2
2	19	F	no	1.5	0.5	2.	9	3	1	1	5
3	53	F	yes	4.5	4	7.5	14	7	1	7	29
4	51	F	no	4	0.5	4.5	10.	9.5	0	1	8
5	55	F	no	2.5	4	6.5	11	7	2	1	8
6	54	F	no	5	2	7	10	1.5	2	2	9
7	47	F	no	1	5.5	6.5	ND	11.5	ND	2	23
8	20	F	no	1.5	2	3.5	9	2	ND	ND	ND
9	19	F	yes	5	4.5	9.5	13	8.5	ND	1	12
10	48	F	yes	2	1.5	3.5	ND	9.5	0	1	5
11	25	F	yes	4	3	6.5	ND	10.5	0	4	10
12	24	M	no	3.5	2.5	6.	7	2.5	0	3	13
13	34	F	no	2	3	5	10	16.5	ND	0	0
14	27	F	yes	2.5	3.5	6	ND	3.5	2.5	1	1
15	39	F	yes	4	2.5	6	ND	11	ND	ND	ND
16	29	F	yes	5.5	3	8	10	9.5	8	1	4
17	34	F	yes	5.5	1.5	7	11	15	1	2	3
18	37	F	yes	4.5	4.5	8.5	9	4.5	1	1	8
19	53	F	no	2	0	2	10	6.5	0	2	2
20	48	F	no	1.5	3	4	8	4	ND	ND	ND
21	50	F	yes	2	0	2	12	7	ND	ND	ND
22	23	F	yes	1.5	3	4	11	8	ND	ND	ND
23	58	F	yes	4	3	7	9	4	ND	ND	ND
24	32	F	no	2	1.5	3.5	9	4.5	ND	ND	ND
25	21	F	yes	2	0.5	2	11	10	ND	ND	ND
26	52	F	yes	2	0.5	2.5	ND	5.5	ND	ND	ND
27	50	F	yes	6	1	7	9	13	ND	ND	ND
28	54	F	no	3.5	1	4.5	9	9.5	ND	ND	ND
29	43	F	no	5.5	2.5	8	12	2.5	ND	ND	ND
30	37	F	yes	3	2	5	10	2.5	ND	5	13
31	36	M	yes	1.5	2	3.5	9	2.5	ND	1	0
32	38	M	no	2	3	4.5	7	8	ND	0	0
33	44	M	no	1.5	0.5	2	9	6.5	ND	0	1
34	25	F	no	0.5	2	2.5	8	4.5	ND	0	6
35	53	M	no	1.5	2	3.5	8	2	ND	0	2
36	24	M	yes	4.5	2	6.5	8	2	ND	1	1
37	31	M	yes	3.5	0.5	4	9	3	ND	0	2
38	36	M	no	4	2.5	6	8	2	ND	1	3
39	50	M	yes	3	1.5	4.5	ND	6	ND	2	4
40	55	M	no	2.5	3.5	6	25	7.5	ND	ND	ND
41	51	M	yes	1.5	4.5	6	ND	11	ND	ND	ND
42	49	M	yes	0.5	1	1.5	8	9.5	ND	ND	ND
43	42	M	no	1	2.5	3.5	10	6.5	ND	ND	ND
44	37	M	no	2	1	3	ND	5.5	ND	ND	ND
45	25	M	yes	1	1.5	2.5	10	3.5	ND	ND	ND
46	26	M	yes	2.5	2	4.5	ND	6.5	ND	ND	ND
47	26	M	no	0.5	0.5	1	8	5	ND	ND	ND
48	23	M	yes	1	3	4	8	8	ND	ND	ND
49	20	M	no	1	0	1	11	2.5	ND	ND	ND
50	44	M	no	0.5	0.5	1	8	5	ND	ND	ND
51	41	M	no	1	0.5	1.5	10	2	ND	ND	ND
52	39	M	yes	0.5	1.5	2	9	9.5	ND	ND	ND
53	32	M	yes	1.5	0.5	2	8	14	ND	ND	ND
54	24	M	no	1.5	1	2.5	8	3.5	ND	ND	ND
55	23	M	yes	1.5	0.5	1.5	10	14.5	ND	ND	ND
56	21	M	yes	0.5	1	1.5	8	11.5	ND	ND	ND
57	21	M	no	1.5	1	2.5	8	8	ND	ND	ND
58	20	M	no	3	1.5	4.5	8	11	ND	ND	ND

* Individuals Nos. 1-29 are from factory I and Nos. 40-58 are from factory II. Individuals Nos. 1-18 and 40-49 were exposed to ethylene oxide; Nos. 19-39 and 50-58 were controls, see Materials and methods

menopausal complaints, 4 took other drugs without any known mutagenic activity. Nine were smokers.

Controls I A. — These controls were 11 women aged 21–58 (mean 44) years, who worked in another department of the same factory, without exposure to ethylene oxide. Four took hormones, 4 took other drugs. One person had a herpes zoster infection half a year before the examination. Three persons had had X-ray examinations. One person had until 1.5 year before the investigation been employed in a shop selling various chemicals. Six were smokers. A bone marrow sample was obtained from 1 of these controls.

Controls I B. — When comparing the bone marrow parameters a group of 10 bus drivers (8 males and 2 females), described in detail elsewhere (HÖGSTEDT et al. 1981), were employed.

3.2 Factory II

Working conditions. — In this plant the medical equipment was treated with a mixture (11:89 w/w) of ethylene oxide and Freon®12 in three sterilizers. These were unloaded twice a day during approximately half an hour. After sterilization the equipment was taken by truck to a ventilated stockroom, where it was stored for 10 days before packing in another room. The sterilizing personnel had never worn breathing protective devices. Eight of 10 persons worked partly with truck driving (1–2 h/day) and partly with packing. During truck driving they were exposed to ethylene oxide in the stockroom.

Exposed persons. — These consisted of 10 males aged 20 to 55 (mean 36) years, who had been exposed for 0.5 to 8 (mean 1.7) years. Seven of them (all working with truck driving and packing) had been employed just a little more than half a year before the investigation, 2 for about a year and 1 for 8 years. In summary, at biological sampling, 2 subjects were exposed to 8 h TWA levels of 1–2 ppm. One of them had until 2 years before sampling been exposed to considerably higher levels. The others were exposed to levels below 0.5 ppm. One subject had previously been exposed occupationally to inorganic lead until one year before the investigation. He had a blood lead value of 0.17 mg/l. Eight subjects had had X-ray examinations and 1 person took thyroxine because of hypothyreosis. Five were smokers.

Controls II. — Nine male workers from another department of the same factory, who were not exposed to ethylene oxide, were used as controls.

Their age ranged from 20 to 44 (mean 29) years. One of them had possibly had mononucleosis half a year before the investigation, and 1 had had parotitis three months before. Four persons had had X-ray examinations. None of them took any drug. Four were smokers.

4. Cytogenetic methods

4.1 Peripheral blood lymphocytes

Chromosome aberrations. — The same standard microculture technique was used as in previous studies by our group (e.g., HÖGSTEDT et al. 1979). The cultivation time was 72 h. Preparations were coded and analyzed by one observer without any knowledge of the exposure. Two hundred metaphases were scored from each individual and the aberrations were recorded according to the classification system recommended by ISCN (1978). The chromosomal aberrations were referred to either as 'breaks', i.e. chromatid breaks, chromatid interchanges, acentric fragments, ring chromosomes, dicentric chromosomes, and pericentric inversions, or as 'gaps' which included both chromatid and isochromatid gaps. Agreement with another observer was required for scoring a cell as abnormal; observed cells were never rejected. Samples from exposed and control individuals were prepared and analyzed in the same way.

Sister chromatid exchanges (SCE). — The frequency of SCE was studied by the Giemsa-technique essentially according to WOLFF and PERRY (1974). The lymphocytes were stimulated with phytohaemagglutinin and cultivated for 72 h in 5-bromodeoxyuridine at a final concentration of 0.1 mM/ml medium. Twenty metaphases with 46 chromosomes were studied from each individual on coded slides by one observer.

Micronuclei. — The same preparations as for chromosome analysis were used. Two thousand lymphocyte nuclei were identified and the frequency of micronuclei was scored. The micronuclei were of different sizes and before accepting them as micronuclei they were compared with the main nucleus regarding size, structure and color; they had to be not larger than approximately one third of the main nucleus and if they were larger than a small lymphocyte nucleus they had to have almost the same color and structure as the main nucleus. They had to be situated within two nuclear diameters from the main nucleus but clearly separate from it. The preparations were examined with an oil immersion lens (magnification $\times$

1,000). This is essentially the same method as that described by COUNTRYMAN and HEDDLE (1976).

4.2 Bone marrow cells

Chromosome aberrations. — The bone marrow cells used for chromosome studies were obtained from the same sample as the cells for the micronucleus test. The cells, suspended in isotonic phosphate buffer (pH 7) were centrifuged a few hours after collection and incubated overnight in McCoy's medium without any mitogen stimulation. Otherwise, preparations were made in the same manner as for lymphocytes. Twenty-five to 100 mitoses from each individual were analyzed. The aberrations were classified according to the same system as that used for the aberrations in lymphocytes. Gaps were, however, not recorded.

Micronuclei. — Bone marrow cells were obtained by sternal puncture at the same time as the blood samples. The marrow cells were prepared according to the micronucleus test method described by SCHMID (1975) as modified by JENSSEN and RAMEL (1976). The cells were suspended in a mixture of equal parts of isotonic phosphate buffer with pH 7 and fetal calf serum. After vigorous shaking, the cells were pelleted and the supernatant removed. The pellet was resuspended in a few drops of buffer and calf serum solution. The cells were smeared on glasses, air-dried, and then the smears were stained with May-Grünwald-Giemsa's stain.

Only interphase cells were analyzed. The micronucleus in a polychromatic erythrocyte was defined as a rounded, bluish or almost black, sharply outlined structure, about $1\mu\text{m}$ across. The micronucleus in an erythroblast was somewhat larger and of the same color as the main nucleus. For each individual 1,000 erythroblasts and 1,000 polychromatic erythrocytes were examined by one observer.

5. Statistical methods

To evaluate the effects of exposure against other types of possible influences on the different parameters expressing cytogenetic damage (chromosomal aberrations, SCE and micronuclei in lymphocytes; chromosomal aberrations and micronuclei in bone marrow cells) analysis of covariance was used (LINDMAN 1974). This analysis included age, smoking, ionizing radiation, drug intake, exposure to ethylene oxide and also the belonging to a certain factory or subgroup of the

material. Because of the unequal proportions of men and women in the different subgroups of the material sex was not possible to include in the analysis of covariance. However, sex is not known to be a predisposing factor to cytogenetic damage (GALLOWAY and EVANS 1975; LATT and JUERGENS 1977; WAKSVIK et al. 1981). Since analysis of covariance demands a normal distribution of the variables the values were transformed into logarithms, which approximately met this requirement. The Spearman rank correlation test was used for the analysis of correlation (SIEGEL 1956, p. 203–213). All tests were two-sided. Statistical significance denotes $P < 0.05$.

Results

Exposed vs. control subjects

Exposed subjects in factory I had higher mean frequencies of cells with breaks, gaps and total number of chromosome aberrations in peripheral blood lymphocytes (Table 2 and 3; Fig. 1 and 2) than controls from the same factory. Also, exposed persons in factory II had higher mean frequencies of cells with breaks, gaps and total number of chromosome aberrations than corresponding controls. Analysis of covariance revealed that the total number of chromosome aberrations and gaps showed significant effect of exposure (Table 3), when other possible background influences (age, smoking habits, etc.) were eliminated. This was, however, not true for breaks.

Neither the frequencies of SCE, nor of micronuclei in lymphocytes showed any significant effect of exposure.

In bone marrow, exposed subjects had significantly higher levels of micronuclei in erythroblasts and in polychromatic erythrocytes (Tables 2 and 3; Fig. 1 and 2).

There was a significant effect of smoking on the frequencies of micronuclei in lymphocytes and in erythroblasts ($P = 0.007$ and 0.04 , respectively). With the other cytogenetic parameters smokers generally had higher values than non-smokers, but the differences were not significant.

Associations between different cytogenetic parameters

Twenty-six correlation coefficients were calculated on the different cytogenetic parameters and are presented in Table 4. Out of 23 positive coeffi-

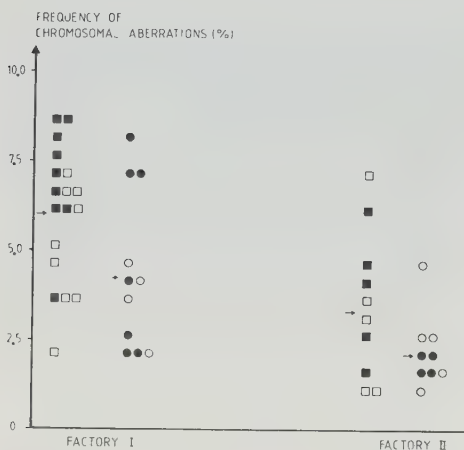


Fig. 1. Chromosomal aberrations in persons exposed to ethylene oxide (squares) and controls (dots). Smokers have closed and non-smokers open symbols. The mean values are indicated by arrows.

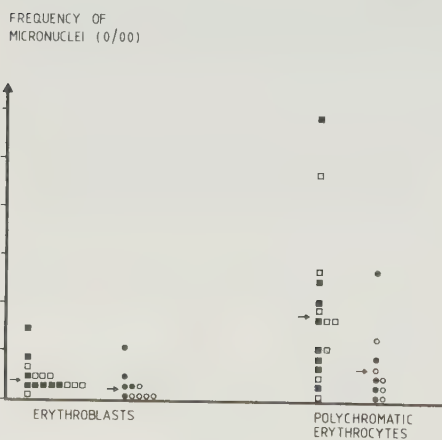


Fig. 2. Micronuclei in erythropoietic bone marrow cells in persons exposed to ethylene oxide (squares) and controls (dots). Smokers have closed and non-smokers open symbols. The mean values are indicated by arrows.

cients 6 were statistically significant with two-sided P values less than 0.05. Three coefficients were negative but not significant. Thus, positive and significant correlations existed between: chromosome aberrations in lymphocytes and bone marrow cells; gaps and breaks in lymphocytes; breaks and SCE in lymphocytes; chromosome

Table 3. Summary of background data and results of cytogenetic tests as well as statistical testing by covariance in persons exposed to ethylene oxide and controls

						Peripheral blood lymphocytes				Bone marrow cells with				
No. of individuals	Mean age	No. of smokers	No. of persons with drug intake	No. of X-ray exposed persons	Cells with chromosomal aberrations				SCE	Micro-nuclei	Chromo-somal aberrations (%)	Micronuclei in		
					Breaks (%)	Gaps (%)	Total (%)	Mean No./cell				Erythro-blasts (%)	Polychromatic erythrocytes (%)	
Factory I														
18 exposed controls	37	9	8	5	3.4	2.7	6.0	10.2	1.4	1.9	8.8			
11 controls	44	6	7	6	2.9	1.5	4.2	10.0	6.8	—	—			
10 controls	37	5	2	4	—	—	—	—	—	1.0	3.2			
Factory II														
10 exposed controls	36	5	1	7	1.4	2.0	3.3	11.4	6.6	—	—			
9 controls	29	4	1	4	1.3	0.9	2.1	8.5	8.8	—	—			
Two-sided P-values (exposed/controls)						>0.05	0.004	0.008	> 0.05	—	0.050	0.030		

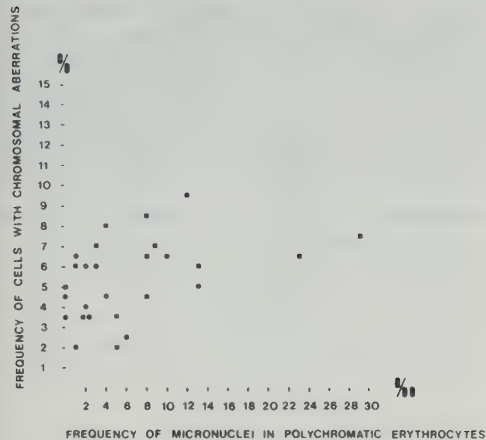


Fig. 3. Association between the frequencies of total chromosomal aberrations in peripheral blood lymphocytes and micronuclei in polychromatic erythrocytes of the bone marrow ($r = 0.47$, $P = 0.014$, $n = 27$) in persons exposed to ethylene oxide (squares) and controls (dots). Smoking habits are not indicated.

aberrations in lymphocytes and micronuclei in polychromatic erythrocytes (Fig. 3); breaks in lymphocytes and micronuclei in erythroblasts; and micronuclei in polychromatic erythrocytes and erythroblasts.

Discussion

This study has shown that, compared to controls, persons exposed to low levels of ethylene oxide have significantly higher frequencies of chromosomal aberrations in peripheral blood lymphocytes and also of micronuclei in erythropoietic bone marrow cells. Our cytogenetic findings emphasize the potential carcinogenicity of ethylene oxide indicated by other studies (HOGSTEDT et al. 1979a, b; DUNKELBERG 1979).

The differences in cytogenetic damage between exposed individuals and controls regarding chromosomal aberrations in lymphocytes was not large. But it was possible to ascertain statistically significant effects as control groups were carefully

Table 4. Rank correlation coefficients* among different cytogenetic parameters in persons exposed to ethylene oxide and controls

Parameter		1	2	3	4	5	6	7	8
<i>Lymphocytes</i>									
Total aberrations	1	—							
Breaks	2	—	—						
Gaps	3	—		0.30 58 0.020	—				
SCE	4	0.25 48 0.091	0.34 48 0.018	0.08 48 0.48	—				
Micronuclei	5	0.06 58 0.63	0.04 58 0.76	0.02 58 0.86	0.07 47 0.62	—			
<i>Bone marrow</i>									
Breaks	6	0.56 14 0.038	0.39 14 0.17	0.53 14 0.054	0.42 11 0.20	— 0.44 — 13 0.13	—		
Micronuclei in erythroblasts	7	0.36 27 0.064	0.39 27 0.046	0.10 27 0.64	0.41 22 0.060	0.15 26 0.46	— 0.37 — 14 0.19	—	
Micronuclei in polychromatic erythrocytes	8	0.47 27 0.014	0.31 27 0.12	0.35 27 0.076	0.40 22 0.064	0.13 26 0.53	— 0.14 — 14 0.62	0.62 27 0.002	—

* The rank correlation coefficient, the number of individuals and the two-sided P-value are given for each possible coefficient

chosen and influences from suspected cytogenetically active factors were eliminated in the analysis.

An interesting observation is the difference in the levels of chromosome aberrations in lymphocytes between the two factories. This difference was present both in controls and exposed persons and had to be considered in the statistical analysis. The difference may be caused by factors related to the culturing of lymphocytes, to socio-economic differences, or by other unidentified factors.

The exposure to ethylene oxide in the two factories was low. According to the admittedly limited data on hand, all 28 exposed persons had been exposed to TWA-levels not exceeding 1 ppm during the last 2.5 years. Thirteen of them had until 2.5 years before the investigation been exposed to higher levels, but probably not exceeding 5 ppm.

The present results showed low but statistically significant coefficients of correlation between several of the parameters studied. There are 26 possible correlation coefficients and 23 were positive. Six of these were statistically significant, which should be compared with the expected frequency of approximately one significant coefficient. This finding shows that there is a non-random association between the cytogenetic parameters. However, the associations are not close. As discussed elsewhere (HÖGSTEDT et al. 1981) this may have several reasons such as: low precision of the determinations and/or different effect and time sequence of the mutagenic agent on the different parameters studied.

The study of bone marrow cells has advantages: The cells are exposed in all phases of the cell cycle; the cells have a rapid turnover and thus preferentially show effects of recent exposure. In addition the scoring of micronuclei is fast and easy and controls do not need to be sampled simultaneously. However, neither the study of chromosome aberrations nor that of micronuclei will become a routine method, since bone marrow samples are difficult to obtain.

Our results indicate that the analysis of chromosomal aberrations in lymphocytes is a sensitive method. The method is, however, time-consuming. The study of SCE is perhaps more rapid, but we found no significant difference between exposed and control subjects by this parameter. This is in accordance with other comparative studies of chromosome aberrations and SCE after chronic in vivo exposure (BRÖGGER 1982).

Micronuclei in lymphocytes may be scored in the same preparations as those used for chromo-

some aberrations. This analysis is rather simple and very rapid. The method has been employed in some studies, which have shown dose related effects of ionizing radiation and chemical mutagens in vitro (COUNTRYMAN and HEDDLE 1976; HEDDLE et al. 1978; LINNAINMAA et al. 1978) and also increased levels in humans after X-ray examinations with iodine-containing contrast medium in vivo (NORMAN et al. 1978). It is interesting to note that in this parameter there was a statistically significant difference between smokers and non-smokers, but no effect of exposure to ethylene oxide nor any inter-correlation with other cytogenetic parameters. As the preparations were intended for chromosome analysis, the culture time was 72 h, while it has been shown that 96 h are necessary to give a maximum of micronuclei (COUNTRYMAN and HEDDLE 1976). Also, since the cytoplasm of the cells has been torn away by the hypotonic treatment, it is difficult to differentiate between real micronuclei and nuclear/cellular debris from other cells. Some micronuclei may also leave the neighbourhood of the main nucleus when the cytoplasm is destroyed. We find the possibility of studying micronuclei in in vivo exposed lymphocytes extremely interesting. Therefore, in future studies a modified technique that leaves the cells intact will be used.

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Literature cited

- ABRAHAM, R. H. 1980. Recent studies with workers exposed to ethylene oxide. — In *The Safe use of Ethylene Oxide: Proceedings of the Educational Seminar*, HIMA report No. 80-4 (Ed. J. F. JORKASKY), Health Industry Manufacturers Association, Washington, D.C., p. 27–38, 211–220
- APPELGREN, L.-E., ENEROTH, G., GRANT, C., LANDSTRÖM, L.-E. and TENGHAGEN, K. 1978. Testing of ethylene oxide for mutagenicity using the micronucleus test in mice and rats. — *Acta Pharmacol. Toxicol.* 43: 69–71
- BROOKES, P. and LAWLEY, P. D. 1961. The alkylation of guanine and guanylic acid. *J. Chem. Soc.* 3923–3928
- BRÖGGER, A. 1982. Application of SCE to public health. — In *Sister Chromatid Exchange*, (Ed. A. A. SANDBERG), Alan Liss, New York, p. 655–673
- CONAN, L., FOUCAULT, B., SIOU, G., CHAIGNEAU, M. and LE MOAN, G. 1979. Contribution à la recherche d'une action mutagène des résidus d'oxyde d'éthylène, d'éthylène glycol et de chloro-2-éthanol dans le matériel plastique stérilisé par l'oxyde d'éthylène. — *Ann. Fal. Exp. Chim.* 72: 141–151
- COUNTRYMAN, P. J. and HEDDLE, J. A. 1976. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. — *Mutat. Res.* 41: 321–332

- DUNKELBERG, H. 1979. On the oncogenic activity of ethylene oxide and propylene oxide in mice. — *Br. J. Cancer* 39: 588–589
- EHRENBERG, L., GUSTAFSSON, Å. and LUNDQVIST, U. 1956. Chemically induced mutation and sterility in barley. — *Acta Chem. Scand.* 10: 492–494
- EHRENBERG, L., HIESCHE, K. D., OSTERMAN-GOLKAR, S. and WENNBERG, I. 1974. Evaluation of genetic risks of alkylating agents: Tissue doses in the mouse from air contaminated with ethylene oxide. — *Mutat. Res.* 24: 83–103
- EMBREE, J. W. and HINE, C. H. 1975. Mutagenicity of ethylene oxide. — *Toxicol. Appl. Pharmacol.* 33: 172–173
- GALLOWAY, S. M. and EVANS, H. J. 1975. Sister chromatid exchange in human chromosomes from normal individuals and patients with ataxia telangiectasia. — *Cytogenet. Cell Genet.* 15: 57–29
- HEDDLE, J. A., LUE, C. B., SAUNDERS, E. F. and BENZ, R. D. 1978. Sensitivity to five mutagens in Fanconi's anemia as measured by the micronucleus method. — *Cancer Res.* 38: 2983–2988
- HÖGSTEDT, C., MALMQVIST, N. and WADMAN, B. 1979a. Leukemia in workers exposed to ethylene oxide. — *J. Am. Med. Ass.* 241: 1132–1133
- HÖGSTEDT, C., ROHLÉN, O., BERNDTSSON, B. S., AXELSON, O. and EHRENBERG, L., 1979b. A cohort study of mortality and cancer incidence in ethylene oxide production workers. — *Br. J. Ind. Med.* 36: 276–280
- HÖGSTEDT, B., HEDNER, K., MARK-VENDEL, E., MITELMAN, F., SCHÜTZ, A., and SKERFVING, S. 1979. Increased frequency of chromosome aberrations in workers exposed to styrene. — *Scand. J. Work Environ. Health* 5: 333–335
- HÖGSTEDT, B., GULLBERG, B., MARK-VENDEL, E., MITELMAN, F., and SKERFVING, S. 1981. Micronuclei and chromosome aberrations in bone marrow cells and lymphocytes of humans exposed mainly to petroleum vapors. — *Hereditas* 94: 179–187
- ISCN. 1978. An international System for Human Cytogenetic Nomenclature. — *Birth Defects: Original Article Series, Vol. XIV, No. 8, The National Foundation, New York*
- JENSSEN, D. and RAMEL, C. 1976. Dose-response at low doses of X irradiation and MMS on the induction of micronuclei in mouse erythroblasts. — *Mutat. Res.* 41: 310–320
- KALLINGS, L. O. 1967. Haematologic studies on persons occupationally exposed to ethylene oxide. — In *International Atomic Energy Agency Report, SM 92/26*, p. 327–334
- LATT, S. A. and JUERGENSEN, L. A. 1977. Determinants of sister chromatid exchange frequencies in human chromosomes. — In *Population Cytogenetics: Studies in Humans*, (Eds. E. B. HOOK, and I. H. PORTER), Academic Press, N.Y., p. 217–236
- LINDMAN, H. R. 1974. Analysis of Variance in Complex Experimental Designs. — *W. H. Freeman, San Francisco*
- LINNAINMAA, K., MERETOJA, T., SORSA, M. and VAINIO, H. 1978. Cytogenetic effects of styrene and styrene oxide on human lymphocytes and *Allium cepa*. — *Scand. J. Work Environ. Health* 4, suppl. 2: 156–162
- NORMAN, A., ADAMS, F. H. and RILEY, R. F. 1978. Cytogenetic effects of contrast media and triodobenzoic acid derivatives in human lymphocytes. — *Radiology* 129: 199–203
- PERO, R. W., WIDEGREN, B., HÖGSTEDT, B. and MITELMAN, F. 1981. In vivo and in vitro ethylene oxide exposure of human lymphocytes assessed by chemical stimulation of unscheduled DNA synthesis. — *Mutat. Res.* 83: 271–289
- PERO, R. W., BRYNGELSSON, T., WIDEGREN, B., HÖGSTEDT, B. and WELINDER, H. 1982. A reduced capacity for unscheduled DNA synthesis in lymphocytes from individuals exposed to propylene oxide and ethylene oxide. — *Mutat. Res.* 104: 193–200
- PFEIFFER, E. H. and DUNKELBERG, H. 1980. Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during fumigation of food-stuffs. — *Food and Cosmet. Toxicol.* 18: 115–118
- RANNUG, U., GÖTHE, R. and WACHTMEISTER, C. A. 1976. The mutagenicity of chloroethylene oxide, chloroacetaldehyde, s-chloroethanol and chloroacetic acid, conceivable metabolites of vinyl chloride. — *Chem. Biol. Interactions* 12: 251–263
- RAPOPORT, I. A. 1948. Dejstvije oksii etilena, gliksida i glikolej na gennye mutatsii. — *Dokl. Akad. Nauk SSSR* 60: 469–472
- SCHMID, W. 1975. The micronucleus test. — *Mutat. Res.* 31: 9–15
- SIEGEL, S. 1956. Nonparametric Statistics for the Behavioral Sciences. — *McGraw-Hill, Kogakusha, Tokyo*
- WAKSVIK, H., MAGNUS, P. and BERG, K. 1981. Effects of age, sex and genes on sister chromatid exchange. — *Clin. Genet.* 20: 449–454
- WOLFF, S. and PERRY, P. 1974. Differential Giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. — *Chromosoma* 48: 341–352

Sister chromatid exchanges and structural chromosome aberrations in relation to smoking in 91 individuals

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Sister chromatid exchanges (SCE) and structural chromosome aberrations were analyzed in peripheral blood lymphocytes and correlated to smoking habits of 91 individuals. There was no difference between smokers and nonsmokers, neither as regards the frequency of SCE, nor for the frequency of structural chromosome aberrations in the total material. In a subgroup exposed to epoxy resins, though, a significantly elevated number of SCE among the smokers was noted. Conversely, smoking did not have any effect as regards SCE among individuals exposed to ethylene oxide—the exposure group with the highest level of SCE. Thus, although there may be an association between smoking habits and the SCE rate in certain populations, this is apparently not a general phenomenon.

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One factor that may influence the rate of cytogenetic damage in man is cigarette smoking. However, the reports on this aspect are contradictory. A chromosome-damaging effect of smoke condensates *in vitro* has been shown (HOPKIN and EVANS 1979), and an increased frequency of chromosome aberrations has been demonstrated in heavy smokers (OBE and HERHA 1978). In recent years the BrdU-staining technique for the analysis of sister chromatid exchange (SCE) rates (LATT 1973; PERRY and WOLFF 1974) has gained much interest as a sensitive method of revealing DNA damage in cells exposed to various genotoxicants (GEBHART 1981; BRÖGGER 1982; LAMBERT *et al.* 1982). LAMBERT *et al.* (1978) first reported an elevated frequency of SCE in smokers. This has been confirmed in some studies (BALA KRISHNA MURTHY 1979; HUSGAFVEL-PURSIAINEN *et al.* 1980; HUSUM *et al.* 1982), whereas others have found no increase in SCE frequency in moderate as well as heavy smokers (HOLLANDER *et al.* 1978; CROSSEN and MORGAN 1980; ARDITO *et al.* 1980; WAKSVIK *et al.* 1981). Various cigarette smoke condensates have been found to induce increased frequencies of SCE in human lymphocytes *in vitro*, but not in Chinese hamster bone marrow cells *in vivo* (MA-DLE *et al.* 1981).

This paper reports an analysis of the outcome of

SCE rates and of structural chromosome aberrations in relation to smoking in 91 individuals.

Material and methods

91 individuals (62 males and 29 females) were studied. 56 individuals were exposed to various potential genotoxicants, such as ethylene oxide (Cases Nos. 1–22), epoxy resins (Cases Nos. 23–39), toluene (Cases Nos. 40–52), petrol products (Cases Nos. 53–59) and ethanol (Cases Nos. 60–65). 35 individuals (Cases Nos. 66–100) had no exposure to any known genotoxicant. The working conditions, types of exposure, etc. for all subjects are described in detail elsewhere (HEDNER *et al.* 1982). There were 47 smokers (mean age 36.3 years) and 44 nonsmokers (mean age 39.3 years). The same Case Nos. (Table 1) were used in the present study as in HEDNER *et al.* (1982).

The frequency of SCE was analyzed in PHA-stimulated peripheral blood lymphocytes cultured for 72 h and studied with Giemsa-technique essentially according to WOLFF and PERRY (1974) as described in detail by HEDNER *et al.* (1982). 5bromodeoxyuridine (BrdU) was present during the entire culture period at a final concentration of

Table 1. Smoking habits and individual values for the cytogenetic parameters studied in 91 individuals

Exposure	Smoker	Case	Number of aberrations/200 cells				Mean No. of SCE/cell
		No.	Gaps	Breaks	Exchanges	Total	
Ethylene oxide	Yes	3	10	11	1	22	13.0
		5	8	8	1	17	13.4
		10	7	10	2	19	10.3
		11	9	9	1	19	9.0
		13	3	14	0	17	11.3
		20	6	2	0	8	8.2
	No	22	2	1	0	3	8.4
		1	6	3	1	10	9.7
		2	4	9	1	14	9.9
		4	4	3	0	7	9.4
		6	1	8	4	13	9.8
		7	8	6	1	15	11.2
		8	1	3	0	4	8.9
		9	2	3	3	8	9.2
		12	5	7	0	12	6.8
		16	3	2	0	5	9.9
		17	1	1	0	2	8.1
		18	0	2	0	2	11.0
		19	7	5	0	12	24.9
		21	5	2	0	7	10.4
Epoxy resins	Yes	24	0	2	0	2	13.4
		26	4	3	0	7	11.3
		28	1	2	0	3	10.4
		30	1	1	0	2	9.1
		34	2	2	0	4	9.9
		35	3	3	1	7	12.2
	No	23	1	1	1	3	8.2
		25	1	2	0	3	8.7
		27	1	3	0	4	12.7
		29	3	2	0	5	7.1
		31	6	2	0	8	6.3
		32	5	3	0	8	9.8
		33	2	3	1	6	6.2
		36	1	2	0	3	6.3
		37	5	3	0	8	9.7
		38	4	1	0	5	5.1
		39	3	5	1	9	8.2
Toluene	Yes	42	5	6	0	11	7.9
		45	3	0	0	3	9.2
		51	3	3	0	6	7.7
		52	7	6	0	13	8.9
	No	46	9	5	0	14	8.8
		48	0	3	0	3	9.1
Petrol products	Yes	53	4	3	0	7	9.5
		57	4	7	2	13	8.4
		58	1	6	1	8	8.8
	No	54	6	5	1	12	6.8
		55	1	2	1	4	9.5
		56	4	1	0	5	8.2
Ethanol	Yes	59	5	9	1	15	8.1
		60	5	2	2	9	10.2
		61	4	4	1	9	11.4
		62	2	1	1	4	9.7
		63	0	3	1	4	10.6
		64	0	2	1	3	13.2
		65	6	3	1	10	9.6

Exposure	Smoker	Case No.	Number of aberrations/200 cells				Mean No. of SCE/cell
			Gaps	Breaks	Exchanges	Total	
Control	Yes	67	0	4	0	4	12.1
		68	6	4	0	10	11.1
		69	8	5	3	16	8.9
		72	1	4	1	6	11.5
		73	2	10	4	16	9.1
		75	2	7	0	9	9.2
		77	2	4	0	6	8.0
		78	1	3	1	5	8.4
		79	2	3	1	6	8.1
		80	0	3	0	3	10.2
		81	2	1	0	3	7.8
		84	3	1	0	4	8.5
		85	0	1	0	1	10.8
		87	3	4	0	7	10.1
		88	1	1	0	2	9.9
		91	3	5	0	8	8.3
		93	1	2	0	3	8.1
		94	4	8	1	13	6.9
		95	3	5	1	9	8.4
		98	1	11	0	12	8.4
		99	0	8	0	8	7.3
	No	66	6	9	5	20	12.2
		70	6	3	1	10	8.1
		71	3	4	0	7	8.6
		74	0	2	2	4	10.4
		76	1	1	1	3	10.0
		82	3	6	0	9	8.4
		83	1	1	0	2	7.8
		86	1	2	0	3	10.2
		89	1	4	0	5	9.4
		90	3	2	0	5	7.2
		92	3	1	0	4	8.1
		96	5	4	0	9	8.0
		97	1	1	1	3	7.1
		100	2	6	0	8	9.0

0.1 mM/ml medium. 20 metaphases with 46 chromosomes from each individual were analyzed on coded slides.

The frequency of structural chromosome aberrations was analyzed in PHA-stimulated peripheral blood lymphocytes cultured for 72 h by a standard microculture technique described in detail previously (HEDNER et al. 1982). 200 metaphases from each individual were analyzed on coded slides. The aberrations were recorded according to the classification system recommended by ISCN (1978).

The Pearson correlation coefficient test was used for the analysis of correlation. Statistical significance denotes $P < 0.05$. Analysis of covariance was used to evaluate the possible influence of age and sex. All tests were two-sided.

Results

Smoking habits and individual values for the cytogenetic parameters studied are presented in Table 1. In the table, gaps include both chromatid and isochromatid gaps; breaks include chromatid breaks and acentric fragments; exchanges include chromatid interchanges, ring chromosomes, dicentric chromosomes and pericentric inversions. The numbers of cells with each aberration type were given for each individual in HEDNER et al. (1982).

There was no difference in the total material between smokers and nonsmokers as regards the frequency of SCE (Fig. 1). Among individuals exposed to epoxy resins, though, smokers had a significantly higher frequency of SCE ($P < 0.05$).

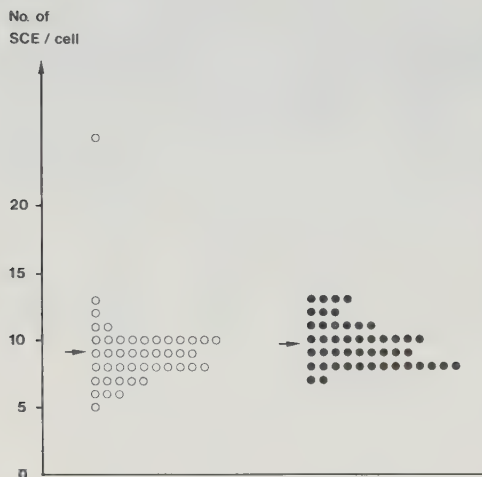


Fig. 1. Association between smoking habits and mean number of SCE/cell in 91 individuals. ● = smokers (mean 9.7), ○ = nonsmokers (mean 9.1).

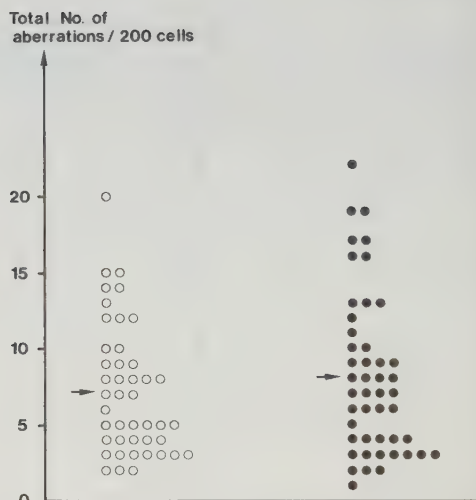


Fig. 2. Association between smoking habits and total number of structural chromosome aberrations/200 cells in 91 individuals. ● = smokers (mean 8.1), ○ = nonsmokers (mean 7.2).

than nonsmokers. Otherwise, no correlations were found between SCE and smoking habits in the different exposure groups, nor among the control subjects.

There was no correlation between the frequency of structural aberrations and smoking habits in the total material (Fig. 2), nor in any of the exposure groups separately or among the controls. In order to eliminate the possible influence of age and sex, an analysis of covariance was performed. Neither, when these genetic background factors were considered, did there appear any effect of smoking on SCE or structural chromosome aberrations in the total material.

Discussion

There was no correlation between SCE frequency and smoking habits in our total material. However, such a correlation was apparent in one particular subgroup: subjects exposed to epoxy resins. This exposure has previously been shown not to have any effect on chromosome aberrations or SCE frequencies when compared to an appropriate control group (MITELMAN et al. 1980). It should be mentioned that smoking did not have

any effect as regards SCE among the ethylene-exposed individuals—the exposure group with the highest level of SCE in this material. This may be in accordance with the report that smoking had no additional adverse effect on SCE in a group of laboratory workers with an elevated number of SCE (LAMBERT et al. 1978).

We found no correlation between the frequency of structural chromosome aberrations and smoking habits in the total material, nor in any of the subgroups. Increased frequencies of structural chromosome aberrations among subjects exposed to ethylene oxide have been reported (HOGSTEDT et al. 1983). Likewise, an increased rate of structural chromosome aberrations has been found in chronic alcoholics (MITELMAN and WADSTEIN 1978). The lack of additional adverse effect of smoking on the ethylene oxide-exposed subjects is in contrast to what has been demonstrated among ethanol-exposed individuals, namely that smoking alcoholics display more chromosome damage than nonsmoking alcoholics (OBE et al. 1980). Studies of the frequency of SCE in chronic alcoholics have, to our knowledge, not been undertaken. In our material of alcoholics no comparison between smokers and nonsmokers could be made, since all the alcoholics were smokers.

Our results indicate that the relationship be-

tween smoking and cytogenetic damage is more complex than originally anticipated. Further data correlating smoking habits to chromosome aberrations and SCE rates in different populations are needed to evaluate the possible effects of this wide-spread environmental factor.

Acknowledgments. — This work was supported by grants from the Swedish Cancer Society, the John and Augusta Persson Foundation for Medical Research, and the Swedish Workers' Protection Fund.

Literature cited

- ARDITO, G., LAMBERTI, L., ANSALDI, E. and PONZETTO, P. 1980. Sister chromatid exchanges in cigarette-smoking human females and their newborns. — *Mutat. Res.* 78: 209–212
- BALA KRISHNA MURTHY, P. 1979. Frequency of sister chromatid exchanges in cigarette smokers. — *Hum. Genet.* 52: 343–345
- BRÖGGER, A. 1982. Application of SCE to public health. — In *Sister Chromatid Exchange* (Ed. A. A. SANDBERG), Alan Liss Inc., New York, p. 655–673
- CROSSEN, P. E. and MORGAN, W. F. 1980. Sister chromatid exchange in cigarette smokers. — *Hum. Genet.* 53: 425–426
- GEBHART, E. 1981. Sister chromatid exchange (SCE) and structural chromosome aberration in mutagenicity testing. — *Hum. Genet.* 58: 235–254
- HEDNER, K., HÖGSTEDT, B., KOLNIG, A.-M., MARK-VENDEL, E., STRÖMBECK, B. and MITELMAN, F. 1982. Relationship between sister chromatid exchanges and structural chromosome aberrations in lymphocytes of 100 individuals. — *Hereditas* 97: 237–245
- HÖGSTEDT, B., GULLBERG, B., HEDNER, K., KOLNIG, A.-M., MITELMAN, F., SKERFVING, S. and WIDEGREN, B. 1983. Chromosome aberrations and micronuclei in bone marrow cells and peripheral blood lymphocytes in humans exposed to ethylene oxide. — *Hereditas* 98: 105–113
- HOLLANDER, D. H., TOCKMAN, M. S., LIANG, Y. W., BORGAONKAR, D. S. and FROST, J. K. 1978. Sister chromatid exchanges in peripheral blood of cigarette smokers and in lung cancer patients; and the effect of chemotherapy. — *Hum. Genet.* 44: 165–171
- HOPKIN, J. M. and EVANS, H. J. 1979. Cigarette smoke condensates damage DNA in human lymphocytes. — *Nature* 279: 241–242
- HUSGAFVEL-PURSIANEN, K., MÄKI-PAKKANEN, J., NORPPA, H. and SORSA, M. 1980. Smoking and sister chromatid exchange. — *Hereditas* 92: 247–250
- HUSUM, B., WULF, H. C. and NIEBUHR, E. 1982. Increased sister chromatid exchange frequency in lymphocytes in healthy cigarette smokers. — *Hereditas* 96: 85–88
- ISCN. 1978. An international system for human cytogenetic nomenclature. — *Cytogenet. Cell Genet.* 21: 309–404
- LAMBERT, B., LINDBLAD, A., NORDENSKJÖLD, M. and WERELIUS, B. 1978. Increased frequency of sister chromatid exchanges in cigarette smokers. — *Hereditas* 88: 147–149
- LAMBERT, B., LINDBLAD, A., HOLMBERG, K. and FRANCESCONI, D. 1982. The use of sister chromatid exchange to monitor human populations for exposure to toxicologically harmful agents. — In *Sister Chromatid Exchange* (Ed. S. WOLFF), John Wiley and Sons, New York, p. 149–182
- LATT, S. 1973. Microfluorometric detection of deoxyribonucleic acid replication in human chromosomes. — *Proc. Nat. Acad. Sci. (USA)* 70: 3395–3399
- MADLE, S., KORTE, A. and OBE, G. 1981. Cytogenetic effects of cigarette smoke condensates in vitro and in vivo. — *Hum. Genet.* 59: 349–352
- MITELMAN, F. and WADSTEIN, J. 1978. Chromosome aberrations in chronic alcoholics. — *Lancet* (1): 216
- MITELMAN, F., FREGERT, S., HEDNER, K. and HILLBERTZ-NILSSON, K. 1980. Occupational exposure to epoxy resins has no cytogenetic effect. — *Mutat. Res.* 77: 345–348
- OBE, G. and HERHA, J. 1978. Chromosomal aberrations in heavy smokers. — *Hum. Genet.* 41: 259–263
- OBE, G., GÖBEL, D., ENGELN, H., HERHA, J. and NATARAJAN, A. T. 1980. Chromosomal aberrations in peripheral lymphocytes of alcoholics. — *Mutat. Res.* 73: 377–386
- PERRY, P. and WOLFF, S. 1974. New Giemsa method for the differential staining of sister chromatids. — *Nature* 251: 156–158
- WAKSVIK, H., MAGNUS, P. and BERG, K. 1981. Effects of age, sex and genes on sister chromatid exchange. — *Clin. Genet.* 20: 449–454
- WOLFF, S. and PERRY, P. 1974. Different Giemsa staining of sister chromatid exchanges without autoradiography. — *Chromosoma* 48: 341–353

Sister Chromatid Exchanges and Structural Chromosome Aberrations in Relation to Age and Sex

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Summary. Sister chromatid exchanges (SCE) and structural chromosome aberrations were analyzed in peripheral blood lymphocytes of 100 individuals, and correlated to age and sex. No correlation was found between the frequency of SCE and age, but older individuals had significantly more structural aberrations than younger. Females had significantly more SCE as well as structural chromosome aberrations than males. The positive correlations of SCE and structural aberrations to age and sex were also significant when these factors, as well as smoking habits, were taken into consideration in an analysis of covariance.

Introduction

Analysis of structural chromosome aberrations in peripheral blood lymphocytes has been used for many years as a means of detecting genetic damage in man. Since the development of the BrdU-staining techniques for the morphologic differentiation of sister chromatids (Latt 1973; Perry and Wolff 1974), analysis of sister chromatid exchange (SCE) rates has become part of the genotoxicology test systems as a potentially more sensitive indicator of DNA damage (Gebhart 1981; Brögger 1982; Lambert et al. 1982). There are, however, few reports of the SCE rate in normal healthy human subjects (Lambert et al. 1976; Morgan and Crossen 1977; Pedersen et al. 1979), and considerable variation in the SCE frequency has been found among the individuals studied. The reason for this may be influences from unknown environmental and/or constitutional host factors. Two genetic factors that may influence the rate of cytogenetic damage are the age and the sex of the subject. In the present study these variables are related to the outcome of SCE rates and of structural chromosome aberrations in 100 individuals.

Material and Methods

One hundred individuals, 69 males aged 18-65 years (mean age 36.4 years) and 31 females aged 18-58 years (mean age 38.9 years) were studied. Sixty five individuals were exposed to various potential genotoxins: ethylene oxide (Cases 1-22), epoxy resins (Cases 23-39), toluene (Cases 40-52), petrol products (Cases 53-59), and ethanol (Cases 60-65). Their working conditions, types of exposure, etc., are described in detail

elsewhere (Hedner et al. 1982). Thirty five individuals (Cases 66-100) had no known such exposure. There were 47 smokers (mean age 36.3 years) and 44 nonsmokers (mean age 39.3 years) in the group; for nine individuals smoking habits were not known. Thirty three males were smokers, 29 were nonsmokers; 14 females were smokers, 15 were nonsmokers.

The frequency of SCE was analyzed in PHA-stimulated peripheral blood lymphocytes cultured for 72 h and studied with Giemsa-technique essentially according to Wolff and Perry (1974) as described in detail by Hedner et al. (1982). 5-Bromo-deoxyuridine (BrdU) was present during the entire culture period at a final concentration of 100 $\mu\text{mol/l}$ medium. Twenty metaphases with 46 chromosomes from each individual were analyzed on coded slides.

The frequency of structural chromosome aberrations was analyzed in PHA-stimulated peripheral blood lymphocytes cultured for 72 h by a standard microculture technique. The procedure is described in detail elsewhere (Hedner et al. 1982). Two hundred metaphases from each individual were analyzed on coded slides. The aberrations were recorded according to the classification system recommended by ISCN (1978).

The Pearson correlation coefficient test was applied for the analysis of correlation. Statistical significance denotes $P < 0.05$. All tests were two-sided. Analysis of co-variance was used to evaluate the possible influence of age, sex and smoking habits on the different cytogenetic parameters.

Results

Age, sex and individual values for the cytogenetic parameters studied are presented in Table 1. In the table, gaps include both chromatid and isochromatid gaps, breaks include chromatid breaks and acentric fragments, exchanges include chromatid interchanges, ring chromosomes, dicentric chromosomes, pericentric inversions and marker chromosomes. The numbers of cells with aberrations for each individual were given in Hedner et al. (1982).

No significant correlation was found between the frequency of SCE and the age of the subjects (Fig. 1). However, such a correlation was found for the frequency of structural aberrations. Thus, older subjects had significantly more aberrations than younger (Fig. 2a-d). This was true for gaps ($P < 0.05$), breaks ($P < 0.05$), exchanges ($P < 0.05$) and total no. of aberrations ($P < 0.01$).

There was a significant sex difference in SCE rates. Females had higher values than males ($P < 0.01$) (Fig. 3). Females also

Table 1. Age, sex and individual values for the cytogenetic parameters studied in 100 individuals

Case No.	Age	Sex	No. of aberrations/200 cells				Mean No. of SCE/cell
			Gaps	Breaks	Ex-changes	Total	
1	34	F	6	3	1	10	9.7
2	53	F	4	9	1	14	9.9
3	19	F	10	11	1	22	13.0
4	20	F	4	3	0	7	9.4
5	53	F	8	8	1	17	13.4
6	51	F	1	8	4	13	9.8
7	54	F	8	6	1	15	11.2
8	19	F	1	3	0	4	8.9
9	51	F	2	3	3	8	9.2
10	29	F	7	10	2	19	10.3
11	37	F	9	9	1	19	9.0
12	24	M	5	7	0	12	6.8
13	34	F	3	14	0	17	11.3
14	54	F	7	6	1	14	10.9
15	39	F	5	14	0	19	13.2
16	25	M	3	2	0	5	9.9
17	26	M	1	1	0	2	8.1
18	20	M	0	2	0	2	11.0
19	55	M	7	5	0	12	24.9
20	23	M	6	2	0	8	8.2
21	42	M	5	2	0	7	10.4
22	49	M	2	1	0	3	8.4
23	21	M	1	1	1	3	8.2
24	25	F	0	2	0	2	13.4
25	32	M	1	2	0	3	8.7
26	26	M	4	3	0	7	11.3
27	18	F	1	3	0	4	12.7
28	41	M	1	2	0	3	10.4
29	58	F	3	2	0	5	7.1
30	35	M	1	1	0	2	9.1
31	59	M	6	2	0	8	6.3
32	55	M	5	3	0	8	9.8
33	65	M	2	3	1	6	6.2
34	34	M	2	2	0	4	9.9
35	35	F	3	3	1	7	12.2
36	29	M	1	2	0	3	6.3
37	53	M	5	3	0	8	9.7
38	29	M	4	1	0	5	5.1
39	50	M	3	5	1	9	8.2
40	47	M	1	7	0	8	9.1
41	18	M	2	0	1	3	8.7
42	32	M	5	6	0	11	7.9
43	44	M	2	7	0	9	7.6
44	18	M	4	4	1	9	8.8
45	36	M	3	0	0	3	9.2
46	56	M	9	5	0	14	8.8
47	18	M	5	2	0	7	9.3
48	30	M	0	3	0	3	9.1
49	28	M	1	2	0	3	7.8
50	18	M	5	2	1	8	8.2
51	32	M	3	3	0	6	7.7
52	37	M	7	6	0	13	8.9
53	46	M	4	3	0	7	9.5
54	48	M	6	5	1	12	6.8
55	44	M	1	2	1	4	9.5
56	25	F	4	1	0	5	8.2
57	24	M	4	7	2	13	8.4
58	31	M	1	6	1	8	8.8
59	36	M	5	9	1	15	8.1
60	49	M	5	2	2	9	10.2
61	51	M	4	4	1	9	11.4
62	41	M	2	1	1	4	9.7
63	50	M	0	3	1	4	10.6
64	32	M	0	2	1	3	13.2
65	38	M	6	3	1	10	9.6
66	43	F	6	9	5	20	12.2
67	50	F	0	4	0	4	12.1
68	23	F	6	4	0	10	11.1
69	58	F	8	5	3	16	8.9
70	48	F	6	3	1	10	8.1
71	31	F	3	4	0	7	8.6
72	21	F	1	4	1	6	11.5
73	50	F	2	10	4	16	9.1
74	53	F	0	2	2	4	10.4
75	53	F	2	7	0	9	9.2
76	41	M	1	1	1	3	10.0
77	24	M	2	4	0	6	8.0
78	32	M	1	3	1	5	8.4
79	21	M	2	3	1	6	8.1
80	23	M	0	3	0	3	10.2
81	21	M	2	1	0	3	7.8
82	20	M	3	6	0	9	8.4
83	44	M	1	1	0	2	7.8
84	38	M	3	1	0	4	8.5
85	21	F	0	1	0	1	10.8
86	27	M	1	2	0	3	10.2
87	33	M	3	4	0	7	10.1
88	28	M	1	1	0	2	9.9
89	41	M	1	4	0	5	9.4
90	49	F	3	2	0	5	7.2
91	56	M	3	5	0	8	8.3
92	35	M	3	1	0	4	8.1
93	59	M	1	2	0	3	8.1
94	56	M	4	8	1	13	6.9
95	29	M	3	5	1	9	8.4
96	50	M	5	4	0	9	8.0
97	29	M	1	1	1	3	7.1
98	28	M	1	11	0	12	8.4
99	45	M	0	8	0	8	7.3
100	38	M	2	6	0	8	9.0

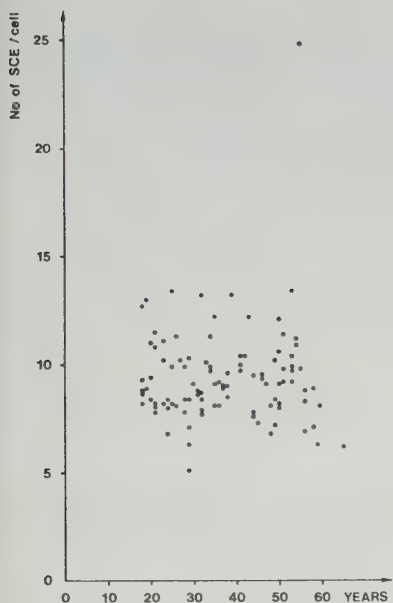


Fig. 1. Association between age and mean no. of SCE/cell in 100 individuals

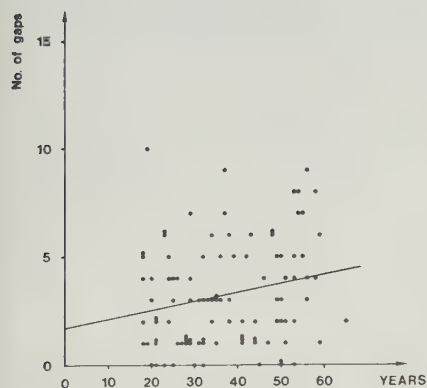
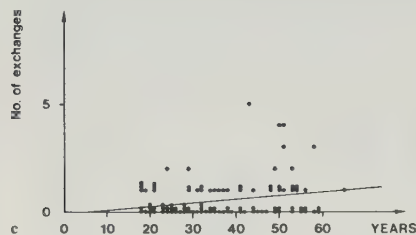
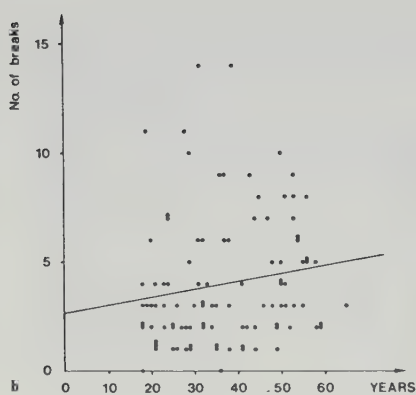


Fig. 2a

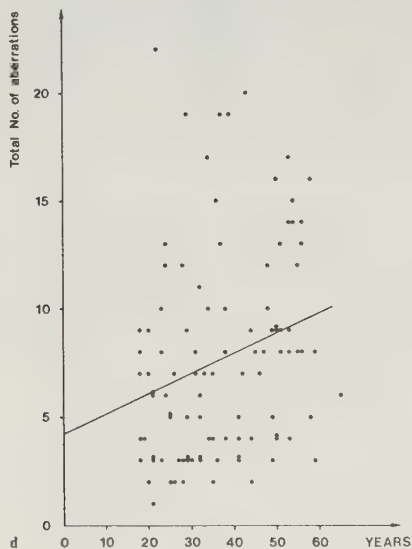


Fig. 2a-d. Association and regression line between age and a no. of gaps, b no. of breaks, c no. of exchanges, d total no. of aberrations in 100 individuals

had significantly more structural aberrations than males (Fig. 4). This was true for breaks ($P < 0.01$), exchange types of aberrations ($P < 0.01$) and total no. of aberrations ($P < 0.01$).

The correlations of SCE and structural chromosome aberrations to age and sex were also significant when these factors, as well as smoking habits, were taken into consideration in an analysis of covariance.

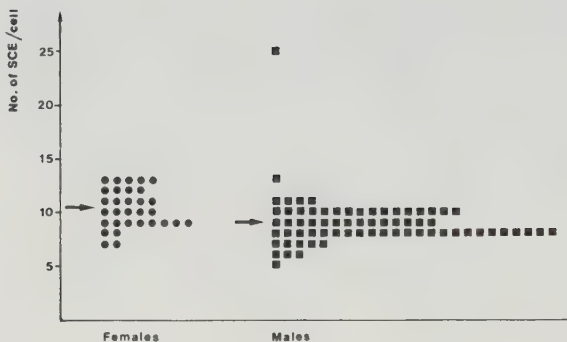


Fig. 3. Association between sex and mean no. of SCE/cell. ● = females (mean = 10.4), ■ = males (mean = 9.0)

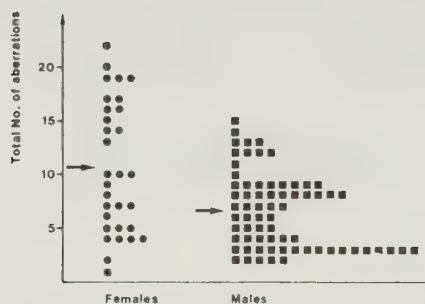


Fig. 4. Association between sex and total no. of aberrations/200 cells. ● = females (mean = 10.6), ■ = males (mean = 6.6)

Discussion

Analyses of structural chromosome aberrations and SCE have proved to be sensitive methods for revealing DNA damage in different *in vitro* systems and also for detecting exposure to certain genotoxic agents *in vivo* (Hollstein et al. 1979). However, very little information is available as regards the possible influences of background genetic factors, e.g. age and sex, on these two types of cytogenetic damage *in vivo*.

In our group of adult subjects there was no correlation between the frequencies of SCE and age. This is in accordance with several previous reports (Galloway and Evans 1975; Funes-Cravioto et al. 1977; Hollander et al. 1978). However, children are reported to have fewer SCE than adults (Funes-Cravioto et al. 1977; Ardito et al. 1980), and recently higher SCE rates have also been found in older individuals (Goh 1981; Schmidt and Sanger 1981; Waksvik et al. 1981). In the experiments of Schneider and Gilman (1979), in which SCEs were studied in human fibroblast cultures after exposure to mitomycin C and *N*-acetoxy-2-acetylaminofluorene, it is interesting in this context to note that, at equal levels of early *in vitro* passage, significantly fewer SCEs were found in cultures from old subjects than in those from young subjects. In contrast, a significant increase in SCE frequency was observed during long term culture at the end of the life span of *in vitro* cultures of normal human fibroblasts (Kato and Stich 1976).

We found a significant positive correlation between incidence of structural chromosome aberrations and age. This is of interest since, even though correlation between age and aneuploidy has been reported occasionally (Jarvik et al. 1976; Fitzgerald and McEwan 1977), there has been, to our knowledge, only one previous finding of correlation between age and structural chromosome aberrations among adults (Tough et al. 1970). However, as with SCE, children have been reported to have fewer chromosome aberrations than adults (Funes-Cravioto et al. 1977). In contrast, neonates have been found to have an increased frequency of chromosome aberrations, falling to adult values within 1 or 2 years (Sasaki et al. 1970).

We found a significant difference between the sexes. Both SCE and structural chromosome aberrations were higher in females. With the aid of covariance analysis it was possible to show that the correlations between the different types of cytogenetic damage and age and sex, respectively, were not interrelated, nor could they be explained by different smoking habits. The relationship between sex and cytogenetic damage has been investigated by several authors, but no sex difference has been noted for SCE (Galloway and Evans 1975; Latt and Juergens 1977; de Arce 1981; Waksvik et al. 1981; Husum et al. 1982), nor for structural chromosome aberrations (Gebhart et al. 1980). It may be mentioned that in several of these studies the values for females were in fact higher than those for males. Apparently these differences were not significant, but may support our findings.

The cause(s) of the sex difference that we have observed can only be speculated upon at present. For example, an increased rate of SCE has been reported in oral contraceptive users (Bala Krishna Murthy and Prema 1979), and therefore other factors than genetic may be considered as well.

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References

- de Arce MA (1981) The effect of donor sex and age on the number of sister chromatid exchanges in human lymphocytes growing *in vitro*. *Hum Genet* 57: 83-85
- Ardito G, Lamberti L, Ansaldi E, Ponzetto P (1980) Sister chromatid exchanges in cigarette-smoking human females and their newborns. *Mutat Res* 78: 209-212

- Bala Krishna Murthy P, Prema K (1979) Sister chromatid exchanges in oral contraceptive users. *Mutat Res* 68:149-152
- Brögger A (1982) Application of SCE to public health. In: Sandberg AA (ed) *Sister chromatid exchange*. Alan Liss Inc New York, pp 655-673
- Fitzgerald P, McEwan Ch (1977) Total aneuploidy and age-related sex chromosome aneuploidy in cultured lymphocytes of normal men and women. *Hum Genet* 39:329-337
- Funes-Cravioto F, Kolmodin-Hedman B, Lindsten J, Nordenskjöld M, Zapat-Gayon C, Lambert B, Norberg E, Olin R, Swensson Å, (1977) Chromosome aberrations and sister chromatid exchange in workers in chemical laboratories and a rototyping factory and in children of women laboratory workers. *Lancet* 2:322-325
- Galloway SM, Evans HJ (1975) Sister chromatid exchange in human chromosomes from normal individuals and patients with ataxia telangiectasia. *Cytogenet Cell Genet* 15:17-29
- Gebhart E, Lösing J, Wopfner F (1980) Chromosome studies on lymphocytes of patients under cytostatic therapy. I. Conventional chromosome studies in cytostatic interval therapy. *Hum Genet* 55:53-63
- Gebhart E (1981) Sister chromatid exchange (SCE) and structural chromosome aberration in mutagenicity testing. *Hum Genet* 58:235-254
- Goh K (1981) Sister chromatid exchange in the aging population. *J Med* 12:195-198
- Hedner K, Högstedt B, Kolnig AM, Mark-Vendel E, Strömbeck B, Mitelman F (1982) Relationship between sister chromatid exchanges and structural chromosome aberrations in lymphocytes of 100 individuals. *Hereditas* 97:237-245
- Hollander DH, Tockman MS, Liang YW, Borgeonkar DS, Frost JK (1978) Sister chromatid exchanges in peripheral blood of cigarette smokers and in lung cancer patients; and the effect of chemotherapy. *Hum Genet* 44:165-171
- Hollstein M, McCann J, Angelosanto FA, Nichols WW (1979) Short-term tests for carcinogens and mutagens. *Mutat Res* 65:133-226
- Husum B, Wulf HCh, Niebuhr E (1982) Increased sister chromatid frequency in lymphocytes in healthy cigarette smokers. *Hereditas* 96:85-88
- ISCN (1978) An international system for human cytogenetic nomenclature. *Cytogenet Cell Genet* 21:309-404
- Jarvik LF, Yen FS, Fu TK, Matsuyama SS (1976) Chromosomes in old age: a six year longitudinal study. *Hum Genet* 33:17-22
- Kato H, Stich HF (1976) Sister chromatid exchanges in ageing and repair-deficient human fibroblasts. *Nature* 260:447-448
- Lambert B, Hansson K, Lindsten J, Sten M, Werelius B (1976) Bromodeoxyuridine-induced sister chromatid exchanges in human lymphocytes. *Hereditas* 83:163-174
- Lambert B, Lindblad A, Holmberg K, Francesconi D (1982) The use of sister chromatid exchange to monitor human populations for exposure to toxicologically harmful agents. In: Wolff S (ed) *Sister chromatid exchange*. John Wiley and sons, New York, pp 149-182
- Latt SA (1973) Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc Nat Acad Sci (USA)* 70:3395-3399
- Latt SA, Juergens LA (1977) Determinants of sister chromatid exchange frequencies in human chromosomes. In: Hook EB, Porter IH (eds) *Population cytogenetics: studies in humans*. Academic Press, New York, pp 217-236
- Morgan WF, Crossen PE (1977) The incidence of sister chromatid exchanges in cultured human lymphocytes. *Mutat Res* 42:305-312
- Pedersen C, Olah E, Merrild U (1979) Sister chromatid exchanges in cultured peripheral lymphocytes from twins. *Hum Genet* 52:281-294
- Perry P, Wolff S (1974) New Giemsa method for the differential staining of sister chromatids. *Nature* 251:156-158
- Sasaki MS, Tonomura A, Matsubara S (1970) Chromosome constitution and its bearing on the chromosomal radio-sensitivity in man. *Mutat Res* 10:617-633
- Schmidt MA, Sanger WG (1981) Sister chromatid exchange in aged human lymphocytes. A brief note. *Mechanisms of ageing and development* 16:67-70
- Schneider E, Gilman B (1979) Sister chromatid exchanging and ageing. III. The effect of donor age on mutagen-induced sister chromatid exchange in human diploid fibroblasts. *Hum Genet* 46:57-63
- Tough IM, Smith PG, Court Brown MW, Harndon DG (1970) Chromosome studies on workers exposed to atmospheric benzene. The possible influence of age. *Eur J Cancer* 6:49-55
- Waksvik H, Magnus P, Berg K (1981) Effects of age, sex and genes on sister chromatid exchange. *Clin Genet* 20:449-454
- Wolff S, Perry P (1974) Different Giemsa staining of sister chromatid exchanges without autoradiography. *Chromosoma* 48:341-353

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A twin study of sister chromatid exchanges in human lymphocytes following carcinogen exposure and DNA repair incubation

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Summary

Sister chromatid exchanges (SCE) were analyzed in peripheral lymphocytes from nine monozygotic (MZ) and nine dizygotic (DZ) healthy male twin pairs. Moreover, the outcome of SCE rates following in vitro treatment of whole blood with $100\text{ }\mu\text{mol/l}$ N-acetoxy-2-acethylaminofluorene (NA-AAF), and after an 18 h DNA repair incubation period, were analyzed in the same twin pairs. There was no significant difference in the intraclass correlation coefficients of SCE frequencies among MZ and DZ twin pairs at the baseline level, nor after NA-AAF treatment or after a DNA repair incubation period. It was concluded that genetic factors probably do not contribute a significant degree of the individual variation observed in baseline or NA-AAF induced SCE. Thus, any observed alterations in SCE frequencies are probably caused by environmental influences.

Introduction

Analysis of sister chromatid exchange (SCE) rates has been reported to be a sensitive indicator of DNA damage. However, in man a considerable variation in the baseline SCE frequency has been noted in the various populations studied (Lambert et al. 1975; Kurvink et al. 1978; Bala Krishna Murthy and Prema 1979; Morgan and Crossen 1981; Carrano and Moore 1982). This variation could be explained by methodological factors such as different culture conditions, and/or by unrecognized environmental and genetic background factors. There are few reports dealing with the possible contribution of genetic components to variation in SCE rates between individuals (Pedersen et al. 1979; Waksvik et al. 1981; Lambert et al. 1982). These studies have used twins to assess intraclass variance, and they have shown essentially no genetic influence. In this paper an attempt has been made to evaluate genetic influences on SCE rates by studying baseline SCE rates in peripheral lymphocytes from eighteen male twin pairs. In addition, the outcome of SCE rates immediately after in vitro treatment with N-acetoxy-2-acethylaminofluorene (NA-AAF) and also after 18 h of DNA repair incubation following NA-AAF treatment has been evaluated for heritable factors.

Material and methods

Eighteen male twin pairs were selected from the Swedish Twin Registry. The twins were aged 25-33 years, and were concordant within pairs in their smoking habits and type of occupation. None of them was known to have a viral infection or cancer at the time of blood sampling. In no case was there evidence that occupational exposure may have caused induction of SCE. Both members of each twin pair were sampled on the same day after an overnight fast. Nine twin pairs were monozygotic (MZ) and nine pairs were dizygotic (DZ). Zygosity was determined by clinical examination and verified by a questionnaire method, based on the answers of both members of the twin pair to questions on similarity and confusion in childhood. The validity of this method has been tested by making blood marker determinations of eleven polymorphic marker systems in 104 randomly sampled twin pairs. The agreement between questionnaire and blood marker diagnosis was 100% (Sarna et al. 1978).

A 6 ml fresh peripheral blood sample from each twin was exposed to a $100\mu\text{mol/l}$ dose of NA-AAF for 1 h at 37°C . NA-AAF was dissolved in dimethyl sulfoxide (<1% of blood) before addition to the whole blood samples. A 3 ml unexposed blood sample from the same individual was incubated for 1 h at 37°C at the same time in order to provide an appropriate control sample. After incubation the exposed blood samples were divided into two 3 ml samples, and 10 drops of blood from each sample was cultured for 72 h by the standard microculture technique described in detail elsewhere (Hedner et al. 1982). Phytohemagglutinine (PHA) was added immediately before culture to the control samples and to one of the duplicate NA-AAF exposed samples. The other exposed

blood sample received PHA after 18 h in culture. The frequency of SCE was analyzed with Giemsa-technique essentially according to Wolff and Perry (1974) as described by Hedner et al. (1982). 5-Bromodeoxyuridine (BrdU) was present during the entire culture period of 72 h at a final concentration of $100\text{ }\mu\text{mol/l}$ medium. 20 metaphases (whenever possible) with 46 chromosomes from each individual were analyzed on coded slides.

Student's t-test was used to analyze differences between means. Heritability (h^2) was estimated by the formula $h^2 = 2(r_{MZ} - r_{DZ})$ where r_{MZ} and r_{DZ} are the sample intraclass correlation coefficients among MZ and DZ twins, respectively (Weinberg et al. 1976). For comparison, calculations were made also by the conventional methods of F-test and one-way analysis of variance.

Results

The mean baseline SCE frequency was 8.8 (S.D.=1.6) among the MZ twins and 9.1 (S.D.=1.5) among the DZ twins. The mean SCE frequency after 1 h of NA-AAF treatment was 15.7 (S.D.=2.4) among the MZ twins and 15.6 (S.D.=4.1) among the DZ twins. The mean SCE frequency after 18 h of DNA repair incubation was 14.6 (S.D.=3.0) among the MZ twins and 17.2 (S.D.=2.4) among the DZ twins (table 1).

There was no significant difference in the intraclass correlation coefficients of SCE frequencies among MZ and DZ twin pairs at the baseline level, after NA-AAF treatment or following DNA repair incubation (fig. 1). Neither was there any significant difference in the intraclass variation as regards the difference between the baseline level of SCE rates and the NA-AAF induced SCE rates, between the NA-AAF induced SCE rates and the SCE rates after DNA repair incubation, nor between the baseline level of SCE rates and the SCE rates after DNA repair incubation.

The intraclass correlation coefficients are presented in table 2. Heritability estimates resulted in h^2 -values of 0 at the baseline level, -1.16 after NA-AAF treatment, 0.32 after DNA repair incubation, -1.84 for the difference between the baseline levels and the NA-AAF induced SCE rates, 0.04 for the difference between the NA-AAF induced SCE rates and the SCE rates after DNA repair incubation, and 0.48 for the difference between the baseline levels and the SCE rates after DNA repair incubation.

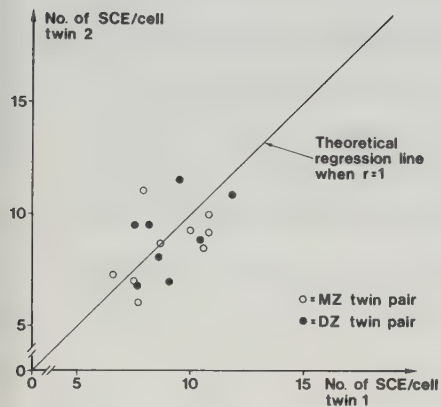
Table 1. Mean No. of SCE/cell at the baseline level, after 1 h of NA-AAF treatment, and after 18 h of DNA repair incubation in eighteen twin pairs.

Zygosity	Twin pair No.	Mean No. of SCE/cell at the baseline level	Mean No. of SCE/cell after 1 h of NA-AAF treatment	Mean No. of SCE/cell after 18 h of DNA repair incubation
MZ	1	10.0	14.7	17.7
		9.3	18.6	17.0
	2	10.8	15.6	12.0
		9.2	19.1	10.1
	3	10.8	11.7	13.5
		10.0	18.0	18.7
	4	10.6	18.8	20.8
		8.5	17.5	16.1
	5	7.9	16.0	17.8
		11.1	17.6	-
	6	7.7	13.9	15.2
		6.1	15.3	13.2
	7	6.6	12.0	13.1
		7.3	15.4	14.3
	8	7.5	13.1	11.1
		7.0	12.1	16.2
	9	8.7	15.8	12.2
		8.7	17.9	12.3
DZ	10	8.2	9.7	15.7
		9.5	14.7	16.2
	11	9.5	20.0	19.1
		11.5	20.1	22.2
	12	11.8	19.8	20.5
		10.9	17.1	14.9
	13	7.6	12.7	-
		6.9	12.7	11.7
	14	7.5	23.0	19.9
		9.5	21.7	-
	15	7.5	13.6	16.9
		-	14.8	16.5
	16	8.6	14.6	16.2
		8.1	17.2	18.2
	17	9.1	10.9	16.5
		7.0	10.6	13.9
	18	10.4	10.5	-
		8.8	16.9	15.8

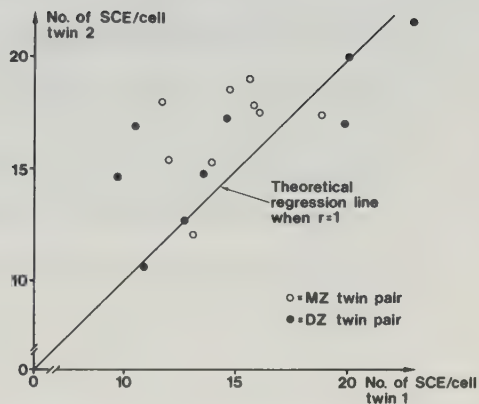
Table 2. Intraclass correlation coefficients at the baseline level (A), after 1 h of NA-AAF treatment (B), after 18 h of DNA repair incubation (C), for the difference between A and B (A'), for the difference between B and C (B'), and for the difference between A and C (C').

	r_{MZ}	r_{DZ}
A	0.52	0.52
B	0.16	0.74
C	0.41	0.25
A'	-0.32	0.60
B'	0.36	0.34
C'	0.46	0.22

a



b



c

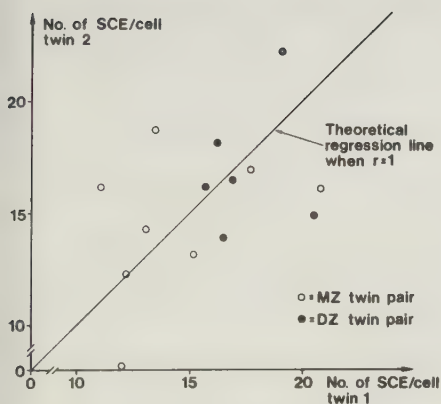


Fig. 1 Intrapair values of mean No. of SCE/cell a) at the baseline level, b) after 1 h of NA-AAF treatment, c) after 18 h of DNA repair incubation. ○ = MZ twin pair, ● = DZ twin pair.

Discussion

A total of 50 twin pairs have been studied so far, and no significant differences in the intraclass variance of SCE frequencies were found between MZ and DZ pairs (Pedersen et al. 1979; Waksvik et al. 1981; Lambert et al. 1982). Our results are in accordance with these previous reports. This apparent lack of genetically determined baseline SCE frequency does not, however, exclude the possibility of genetically determined differences in the response to exposure to mutagenic agents or in the ability of DNA repair. In fact, an individual variation, correlated to differences in age and sex, concerning the potential to accumulate DNA damage following identical carcinogen exposure in vitro has been reported (Pero et al. 1978). The parameters indicating accumulation of DNA damage were in this study a) the stimulation of unscheduled DNA synthesis after induction of DNA damage by NA-AAF, b) the level of NA-AAF induced chromosome aberrations after 8 h of DNA repair incubation, and c) the level of [^3H]7,12-dimethylbenz(a)anthracene (DMBA) bound to DNA after 18 h of incubation of lymphocytes in DMBA. The potential to accumulate DNA damage increased with age for both men and women, while regardless of age, men always had higher values than women. Lately, also some reports on a correlation between SCE and age (Goh 1981; Schmidt and Sanger 1981; Waksvik et al. 1981) and SCE and sex (Hedner et al. 1982) in vivo have appeared. These observations seem to indicate a genetic influence on the sensitivity to carcinogen exposure.

In our material the intrapair variance of the level of NA-AAF induced increase in SCE did not show any significant difference between MZ and DZ twin pairs, nor did the intrapair variance of the SCE frequencies after DNA repair incubation. Thus, when SCE was used as the parameter indicating

DNA damage, a genetically determined sensitivity to carcinogen exposure could not be verified. It may be concluded, then, that any observed increase in SCE frequency is probably caused by environmental influence.

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References

- Bala Krishna Murthy P, Prema K (1979) Sister chromatid exchanges in oral contraceptive users. *Mutat Res* 68:149-152
- Carrano AV, Moore DH (1982) The rationale and methodology for quantifying sister chromatid exchange in human. In: Heddle JA (ed) *Mutagenicity: New horizons in genetic toxicology*. Academic Press, Inc. pp 267-304
- Goh K (1981) Sister chromatid exchange in the aging population. *J Med* 12:195-198
- Hedner K, Högstedt B, Kolnig AM, Mark-Vendel E, Strömbeck B, Mitelman F (1982) Relationship between sister chromatid exchanges and structural chromosome aberrations in lymphocytes of 100 individuals. *Hereditas* 97:237-245
- Hedner K, Högstedt B, Kolnig AM, Mark-Vendel E, Strömbeck B, Mitelman F (1982) Sister chromatid exchanges and structural chromosome aberrations in relation to age and sex. *Hum Genet* 62:305-309
- Kurvink K, Bloomfield CD, Keenan KM, Levitt S, Cervenka J (1978) Sister chromatid exchange in lymphocytes from patients with malignant lymphoma. *Hum Genet* 44:137-144
- Lambert B, Hansson K, Lindsten J, Sten M, Werelius B (1976) Bromodeoxy uridine-induced sister chromatid exchange in human lymphocytes. *Hereditas* 83:163-174
- Lambert B, Lindblad A, Holmberg K, Francesconi D (1982) The use of sister chromatid exchange to monitor human populations for exposure to toxicologically harmful agents. In Wolff S (ed) *Sister chromatid exchange*. John Wiley and sons, New York, pp 149-182

- Morgan WF, Crossen PE (1981) Factors influencing sister chromatid exchange rate in cultured human lymphocytes. *Mutat Res* 81:395-402
- Pedersen C, Oláh E, Merrild U (1979) Sister chromatid exchanges in cultured peripheral lymphocytes from twins. *Hum Genet* 52:281-294
- Pero RW, Bryngelsson C, Mitelman F, Kornfält R, Thulin T, Nordén Å (1978) Interindividual variation in the response of cultured lymphocytes to exposure from DNA damaging chemical agents. *Mutat Res* 53:327-341
- Sarna S, Kaprio J, Sistonen P, Koskenvuo M (1978) Diagnosis of twin zygosity by mailed questionnaire. *Hum Hered* 28:241-254
- Schmidt MA, Sanger WG (1981) Sister chromatid exchange in aged human lymphocytes. A brief note. *Mechanisms of ageing and development* 16:67-70
- Waksvik H, Magnus P, Berg K (1981) Effects of age, sex and genes on sister chromatid exchange. *Clin Genet* 20:449-454
- Weinberg R, Avet LM, Gardner MH (1976) Estimates of the heritability of serum lipoprotein and lipid concentrations. *Clin Genet* 9:588-592
- Wolff S, Perry P (1974) Differential Giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. *Chromosoma* 48:341-353

Sister chromatid exchanges in human lymphocytes after a non-S-phase incubation period to allow excision DNA repair - in vitro exposure to N-acetoxy-2-acethylaminofluorene and ethylene oxide

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Summary

Sister chromatid exchanges (SCE) were analyzed in human peripheral blood lymphocytes at the baseline level, after induction of DNA damage by N-acetoxy-2-acethylaminofluorene (NA-AAF) and ethylene oxide (EO), and after a subsequent 18 h DNA repair incubation period. There was a significant difference between the baseline SCE frequencies as compared to those after 1 h of NA-AAF or EO treatment. There was no significant difference between the SCE frequencies after 1 h of NA-AAF treatment and those after 18 h of DNA repair incubation, suggesting that only a low level of NA-AAF damage to DNA had been removed. However, there was a significant difference between the SCE frequencies after 1 h of EO treatment and those after 18 h of DNA repair incubation, indicating that a significant level of EO induced DNA lesions had been repaired. Thus, it seems likely that the EO induced DNA damage is more easily recognized, and hence more rapidly repaired than the NA-AAF induced damage. The reason for this may be the different chemical nature of the DNA lesions induced, which, in turn, leads to a different kinetics of DNA repair.

Introduction

The induction of sister chromatid exchange (SCE) is S-phase dependent, and hence, it appears to be closely related to DNA replication (Wolff 1982). Although SCE are likely to be related to DNA repair processes, their relationship to repair replication, unscheduled DNA synthesis, and postreplication repair remains unclear (Kato 1977). In an attempt to clarify some aspects of this problem, we have considered evaluating the effect of DNA repair synthesis on the induction of SCE using cultured lymphocytes.

Peripheral lymphocytes are mainly in G_0 -phase (Clarkson and Evans 1972), and it can easily be shown that excision DNA repair synthesis occurs in these cells (Lieberman et al. 1971a, Lieberman et al. 1971b). Therefore, if lymphocytes are cultured after DNA damage induction for 18 h without addition of any mitogen, the lymphocytes would be allowed to remove DNA damage by excision repair before entering S-phase following the addition of mitogen. This general approach has been successfully used with chinese hamster ovary (CHO) cells, which were in proliferative arrest on arginine deficient medium (McRae et al. 1979). CHO cells damaged with UV-irradiation and then arrested for 24 or 48 h before estimation of SCE had significantly lower levels of SCE than the CHO cells not held in arrest.

The implications from this study on CHO cells are that if cells are allowed to repair DNA lesions in the non-S-phase of the cell cycle, there would be less DNA damage present to induce SCE, when the cells are allowed to enter S-phase. In order to further substantiate this hypothesis, we have examined the induction of SCE by two DNA damaging agents, which differ considerably in their kinetics to induce DNA repair synthesis. One carcinogen, N-acetoxy-2-acethylaminofluorene (NA-AAF), induces DNA damage poorly recognized by

cellular repair enzymes, so that there is no observable fast repair component (Ahmed and Setlow 1977, Amacher et al. 1977, Poirier and DeCicco 1977, Pero and Östlund 1980). The other carcinogen, ethylene oxide (EO), is an alkylating agent, reacting mainly with the No. 7 position of guanine (Ehrenberg and Hussain 1981), and this type of DNA damage is rapidly repaired (Regan and Setlow 1974). Our reasoning was that poorly recognized DNA damage would be removed more slowly, and thus the induction of SCE would be less affected by a preincubation period, designed to allow DNA repair before estimation of SCE. Here we report the outcome of SCE frequencies in human peripheral lymphocytes after NA-AAF or EO induced DNA damage and a subsequent 18 h DNA repair incubation period.

Material and methods

A 6 ml fresh peripheral blood sample from each of twenty healthy individuals, all males, was exposed to a $100\text{ }\mu\text{mol/l}$ dose of NA-AAF for 1 h at 37°C , and a 6 ml fresh peripheral blood sample from each of fifteen healthy individuals 11 males and 4 females, was exposed to a 2 mmol/l dose of EO for 1 h at 37°C . NA-AAF was dissolved in dimethyl sulfoxide (<1% of blood), and EO was dissolved in physiologic saline, before addition to the whole blood sample. A 3 ml unexposed blood sample from each individual was incubated for 1 h at 37°C at the same time in order to provide an appropriate control sample. After incubation, the NA-AAF and EO exposed blood samples were divided into two 3 ml samples, and 10 drops of blood from each sample was incubated for 72 h by the standard microculture technique described in detail elsewhere (Hedner et al. 1982). Phytohemagglutinin (PHA) was added immediately before culture to the control samples and to one of the duplicate NA-AAF or EO exposed samples. The other exposed blood sample received PHA after 18 h in culture. The frequency of SCE was analyzed with Giemsa-technique essentially according to Wolff and Perry (1974), with the modifications described by Hedner et al. (1982). 5-Bromodeoxyuridine (BrdU) was present during the entire culture period of 72 h at a final concentration of $100\text{ }\mu\text{mol/l}$ medium. Whenever possible, 20 metaphases with 46 chromosomes from each sample were analyzed on coded slides.

Student's t-test on paired observations was used to analyze differences between means.

Results

The entire SCE data are given in table 1. The mean baseline SCE frequency was 8.4 (S.D.=1.5) among the subjects exposed to NA-AAF, 16.1 (S.D.=3.2) after 1 h of NA-AAF treatment, and 15.7 (S.D.=3.0) after 18 h of DNA repair incubation. There was a significant difference between the baseline SCE frequencies as compared to those after 1 h of NA-AAF treatment ($P<0.001$), and also between the baseline frequencies and those after 18 h of DNA repair incubation ($P<0.001$). However, there was no significant difference between the SCE frequencies after 1 h of NA-AAF treatment and those after 18 h of DNA repair incubation (fig. 1).

The mean baseline SCE frequency was 9.7 (S.D.=1.3) among the subjects exposed to EO, 18.9 (S.D.=3.9) after 1 h of EO treatment, and 16.0 (S.D.=2.2) after 18 h of DNA repair incubation. There was a significant difference between the baseline SCE frequencies as compared to those after 1 h of EO treatment ($P<0.001$), and also between the baseline SCE frequencies and those after 18 h of DNA repair incubation ($P<0.001$). Moreover, there was a significant difference (decrease) between the SCE frequencies after 1 h of EO treatment and those after 18 h of DNA repair incubation ($P<0.001$) (fig. 2).

Table 1. Mean No. of SCE/cell at the baseline level, after 1 h of carcinogen exposure, and after 18 h of DNA repair incubation in lymphocytes of 35 healthy individuals.

Type of exposure	Case No.	Sex	Mean No. of SCE/cell at the baseline level	Mean No. of SCE/cell after 1 h of carcinogen exposure	Mean No. of SCE/cell after 18 h of DNA repair incubation
NA-AAF	1	M	9.3	18.6	17.0
	2	M	9.5	14.7	16.2
	3	M	9.2	19.1	10.1
	4	M	9.5	20.0	19.1
	5	M	11.8	19.8	20.5
	6	M	10.9	17.1	14.9
	7	M	10.6	18.8	20.8
	8	M	8.5	17.5	16.1
	9	M	7.7	13.9	15.2
	10	M	6.1	15.3	13.2
	11	M	6.6	12.0	13.1
	12	M	7.3	15.4	14.3
	13	M	7.5	13.1	11.1
	14	M	7.0	12.1	16.2
	15	M	6.9	12.7	11.7
	16	M	7.5	23.0	19.8
	17	M	8.6	14.6	16.2
	18	M	8.1	17.2	18.2
	19	M	7.0	10.6	13.9
	20	M	8.8	16.9	15.8
EO	21	F	9.6	17.9	15.4
	22	M	8.5	15.9	14.2
	23	M	8.0	18.5	15.0
	24	M	9.4	16.3	14.1
	25	F	10.3	28.4	19.2
	26	M	11.4	24.8	20.8
	27	M	8.4	22.6	18.9
	28	F	9.1	19.4	15.8
	29	M	7.8	16.8	14.1
	30	M	10.4	16.6	14.6
	31	M	9.2	15.5	14.3
	32	F	11.3	15.9	15.1
	33	M	12.1	22.8	17.9
	34	M	10.9	15.8	14.3
	35	M	9.7	16.4	16.0

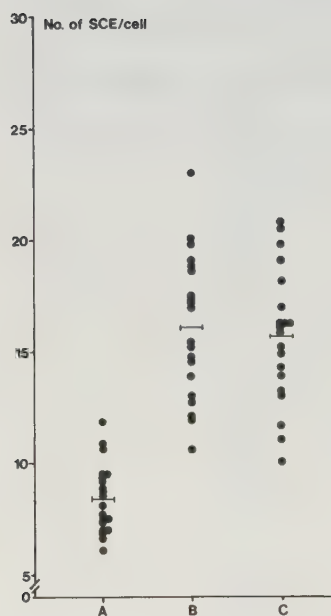


Fig. 1: Mean SCE frequencies at the baseline level (A), after 1 h of NA-AAF exposure (B), and after 18 h of DNA repair incubation (C). T-test: A vs B, $P < 0.001$; B vs C, N.S.; A vs C, $P < 0.001$.

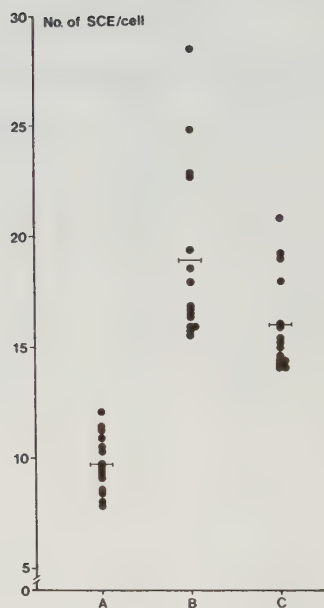


Fig. 2: Mean SCE frequencies at the baseline level (A), after 1 h of EO exposure (B), and after 18 h of DNA repair incubation (C). T-test: A vs B, $P < 0.001$; B vs C, $P < 0.001$; A vs C, $P < 0.001$.

Discussion

There was no decrease in SCE rates in the NA-AAF exposed lymphocytes, when they had been held in non-S-phase for 18 h after NA-AAF exposure (fig. 1), suggesting that only a small portion of the original NA-AAF damage to DNA had been removed. This is in accordance with the hypothesis previously put forward by others (see Introduction), that DNA damage caused by NA-AAF is poorly recognized by the repair enzymes, and hence would not be easily repaired. However, in some of the individuals there was a decrease in SCE frequency, and in this group the mean SCE frequency immediately after NA-AAF exposure was higher than that in the group that did not show a decrease (17.2 versus 14.9). This difference was not statistically significant. However, it may indicate that when more DNA damage is induced, it can be more easily recognized and subsequently repaired, thus giving the result of a reduction in SCE frequency after a DNA repair incubation period.

There was a significant decrease in SCE rates of the EO exposed lymphocytes, when they had been held in non-S-phase for 18 h after EO exposure (fig. 2), indicating that a significant portion of the EO induced DNA damage had been repaired. It seems likely, then, that the EO induced DNA damage is more easily recognized and hence more rapidly repaired than the NA-AAF induced damage, as judged by SCE analysis. The reason for this may be the chemical nature of the DNA lesion. EO ethylates mainly the No. 7 position of guanine in DNA, which increases depurination and destabilizes the phosphodiester bond (Ehrenberg and Hussain 1981). DNA depurinates at neutral pH to some extent even without any induced DNA damage, also causing a similar instability (Lindahl and Nyberg 1972). Hence, an efficient recognition and repair of this type of DNA damage is essential to permit fidelity to the genetic code. NA-AAF

damages DNA primarily in the No. 8 position of guanine, which does not accelerate instability at this site (Kriek 1974), and thus the repair of these DNA lesions are not likely to be controlled by the same repair enzyme(s).

There may be practical implications of these results. For instance, in the field of occupational health care, in cases of exposure to unrecognized carcinogens, that cause SCE alterations, it may be possible to differentiate carcinogens which induce DNA lesions recognized with difficulty by repair enzymes from those that do not.

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